UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

GREGORY MARONEY, individually and on behalf of all others similarly situated, Plaintiff, v. GLANBIA PERFORMANCE NUTRITION, INC. d/b/a SLIMFAST, Defendant.	Civil Action No. **CLASS ACTION COMPLAINT** **JURY TRIAL DEMANDED**

Plaintiff Gregory Maroney ("Plaintiff") brings this action on behalf of himself and all others similarly situated against Defendant Glanbia Performance Nutrition, Inc. d/b/a SlimFast ("Glanbia" or "Defendant"). Plaintiff makes the following allegations pursuant to the investigation of his counsel and based upon information and belief, except as to the allegations specifically pertaining to him, which are based on personal knowledge.

NATURE OF THE ACTION

1. This is a class action lawsuit against Defendant for selling a defective dietary supplement product, SlimFast Keto MCT[1] Oil ("SlimFast MCT" or the "Product"), through its SlimFast brand. The Product contains "100% Pure Coconut Oil" and bears the representation "Clinically Proven Lose Weight & Keep It Off." But that representation is not true. A 2018 peer-reviewed study[2] found "no evidence of difference in…mean weight, BMI, [or] per cent body fat" associated with the use of coconut oil. And far from being "clinically proven" to cause

[1] The acronym "MCT" stands for "medium-chain triglyceride."

[2] Kay-Tee Khaw, et al. *Randomised Trial of Coconut Oil, Olive Oil or Butter on Blood Lipids and Other Cardiovascular Risk Factors in Healthy Men and Women*, 8 BMJ OPEN 1, 9 (2018), https://bmjopen.bmj.com/content/bmjopen/8/3/e020167.full.pdf.

weight loss, a 2015 meta-analysis[3] concluded that "further research is required…to confirm the efficacy of MCT" because "many trials lacked sufficient information" and "commercial bias was detected." Indeed, the American Heart Association[4] "advise[s] against the use of coconut oil" because it is "high in saturated fat…and has no known offsetting benefits." Accordingly, the representations made on the Product's labeling are false and misleading.

2. Plaintiff brings this class action lawsuit on behalf of himself and purchasers of the SlimFast MCT dietary supplements.

BACKGROUND

The Ketogenic Diet

3. "The ketogenic diet is a very low-carb, high-fat diet."[5]

4. The ketogenic diet requires that an individual drastically reduce carbohydrate intake and replace it with fat consumption. This has the effect of changing the body's energy system from relying on carbohydrates and glycogen for energy to relying on fat, usually stored body fat.

5. The ketogenic diet causes weight loss because the body will metabolize stored body fat for fuel. Specifically, fat is converted to ketones, which provide fuel to the body.[6]

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[3] Karen Mumme & Welma Stonehouse, *Effects of Medium-Chain Triglycerides on Weight Loss and Body Composition: A Meta-Analysis of Randomized Controlled Trials*, J. ACAD. NUTRITION AND DIETETICS 249 (2015), https://www.sciencedirect.com/science/article/pii/S2212267214015913.

[4] Frank M. Sacks et al., American Heart Ass'n, *Dietary Fats and Cardiovascular Disease: A Presidential Advisory from the American Heart Association*, 136 CIRCULATION 1 (2017), https://www.ahajournals.org/doi/epub/10.1161/CIR.0000000000000510 (hereinafter "PRESIDENTIAL ADVISORY").

[5] *The Ketogenic Diet: A Detailed Beginner's Guide to Keto*, HEALTHLINE, https://www.healthline.com/nutrition/ketogenic-diet-101#what-it-is (last accessed Feb. 18, 2020)

6. This is a closed feedback loop, and works just fine for the adherent. The problem is that Defendant can't make any money from this system. So Defendant has introduced a line of supplements using the buzzword "Keto", specifically the Product, advertised as a "clinically proven" ingredient to cause weight loss. As will be discussed below, those label claims are false, and Defendant's Product does not cause or accelerate weight loss.

Coconut Oil and Medium-Chain Triglycerides

7. Fatty acids fall within one of three categories: long-chain triglycerides, medium-chain triglycerides, and short-chain triglycerides. Th difference between the three types of fatty acids is the number of carbon atoms they contain. Long-chain triglycerides are the form of fat normally found in food. Medium-chain triglycerides are usually found in coconut oil and certain dairy products.

8. As noted above, SlimFast Keto contains "100% Pure Coconut Oil." "Coconut oil is a highly saturated oil that is traditionally made by extracting the oil from raw coconuts or dried coconut kernels."[7]

9. "Coconut oil is rich in medium-chain fatty acid [MCTs], which are a form of saturated fat."[8] MCTs "are metabolized differently than the long-chain triglycerides (LCT)

(...footnote continued)

[6] https://www.nytimes.com/2020/01/02/style/self-care/keto-diet-explained-benefits.html (last visited 4/2/20).

[7] *Is Coconut Oil Good for Your Skin?*, HEALTHLINE, https://www.healthline.com/nutrition/coconut-oil-and-skin#section1 (last accessed Feb. 18, 2020).

[8] *Id.*

found in most other foods" in that "MCTs go straight to your liver, where they can be used as an instant energy source or turned into ketones."[9]

10. However, MCTs ability to be used as an instant source of energy does not advance the ball in increasing fat loss. For one, Defendant's MCT oil contains 130 calories per serving, and Defendant instructs users to add a serving to "foods or drinks," which can add significant total calories to the user's diet, potentially inhibiting fat loss. Second, the creation of ketones is an effect of fat loss, not a cause. So introducing ketones exogenously inhibits the fat loss process because the body uses fat to make ketones. If ketones are being supplied, the body has less need to burn body fat to create them, which defeats the purpose of the ketogenic diet.

SlimFast MCT Does Not Help With Weight Loss

11. Various peer-reviewed research has determined that these claims that MCTs assist with weight loss are false and misleading.

12. In a 2018 peer-reviewed study,[10] attached as **Exhibit A**, middle-aged men and women "were randomly allocated to consume 50g extra virgin coconut oil or 50g butter or 50g extra virgin olive oil for 4 weeks."[11] At the end of the study, researchers observed "no significant differences in weight or BMI change, [or] change in central adiposity as measured by waist circumference or per cent body fat."[12] There was likewise "no evidence of difference between the groups in mean weight, BMI, per cent body fat[,] or central adiposity at the end of this trial."[13]

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[9] *MCT Oil 101: A Review of Medium-Chain Triglycerides*, HEALTHLINE, https://www.healthline.com/nutrition/mct-oil-101 (last accessed Feb. 18, 2020).

[10] Kay-Tee Khaw, et al., *supra* note 2.

[11] *Id.* at 5.

[12] *Id.* at 5-6

[13] *Id.* at 9.

13. Similarly, a 2015 meta-analysis,[14] attached as **Exhibit B**, "conduct[ed] a systematic review and meta-analysis of randomized controlled trials (RCTs) comparing the effects of MCTs . . . to [long-chain triglycerides] on weight loss and body composition in healthy adults."[15] The meta-analysis reviewed "RCTs . . . published until March 2014."[16] The meta-analysis concluded that "further research is required . . . to confirm the efficacy of MCT."[17]

14. Most poignantly, a Presidential Advisory published by the American Heart Association,[18] attached as **Exhibit C**, "advise[d] against the use of coconut oil."[19] The advisory reviewed "the scientific evidence, including the most recent studies, on the effects of dietary saturated fat intake . . . on [cardiovascular disease]."[20] In discussing coconut oil, the advisory pointed to "7 controlled trials . . . that compared coconut oil with monosaturated or polyunsaturated oils."[21] The advisory noted that "[c]oconut oil raised [low-density lipoprotein] cholesterol in all 7 of these trials, significantly in 6 of them."[22] Low-density lipoprotein is "a leading cause of atherosclerosis,"[23] which is a "disease of the arteries characterized by the

[14] Mumme & Stonehouse, *supra* note 3.

[15] *Id.* at 250.

[16] *Id.*

[17] *Id.* at 249.

[18] PRESIDENTIAL ADVISORY, *supra* note 4.

[19] *Id.* at 14.

[20] *Id.* at 1.

[21] *Id.* at 14.

[22] *Id.*

[23] *Id.* at 2.

deposition of fatty material on their inner walls."[24] The advisory likewise concluded that coconut oil "has no known . . . favorable effects" to offset this increase in low-density lipoprotein.[25]

15. The advisory also noted a major disconnect between the public and nutritionists: where "72% of the American public rated coconut oil as a 'healthy food,'" only "37% of nutritionists" thought the same.[26] The advisory attributed this disparity to the "marketing of coconut oil in the popular press."[27]

16. In conclusion, neither coconut oil nor MCTs are "clinically proven" to help consumers "lose weight and keep it off." Rather, coconut oil is dangerous to the health of consumers, let alone ineffective to assist with weight loss.

SlimFast's Misrepresentations

17. Despite the findings of these various studies, as well as a warning by the American Heart Association, Glanbia continues to misrepresent that its SlimFast MCT product is "Clinically Proven" to help consumers "Lose Weight & Keep It Off" (the "Misrepresentations"). Each of the Misrepresentations appears on the Product's label.

18. Indeed, the front label panel of SlimFast MCT prominently states "Clinically Proven Lose Weight & Keep It Off":

[24] ATHEROSCLEROSIS, LEXICO, https://www.lexico.com/definition/atherosclerosis (last accessed Feb. 18, 2020).

[25] PRESIDENTIAL ADVISORY at 14.

[26] PRESIDENTIAL ADVISORY at 13.

[27] *Id*.

19. The SlimFast MCT Misrepresentations indicate to the consumer that use of the Product will help consumers lose weight and avoid gaining the weight back. But, as discussed above, those label claims are false. In short, the Product does not result in weight loss, and is not clinically proven to lose wight and keep it off.

20. Plaintiff asserts claims on behalf of himself and a nationwide class and New York subclass of purchasers of the Product for violation of New York General Business Law §§ 349

and 350, breach of express warranty, breach of the implied warranty of merchantability, unjust enrichment, and fraud.

THE PARTIES

21. Plaintiff Gregory Maroney is a citizen of New York who resides in Wurtsboro, New York. In or about July 2018, Mr. Maroney purchased a container of Glanbia's SlimFast MCT product for approximately $10 from Wal-Mart in Middletown, New York. Mr. Maroney thereafter purchased more of the Product through October 2019. Mr. Maroney used the Product as directed on the label. The SlimFast MCT supplement he purchased prominently displayed on the package "Clinically Proven Lose Weight & Keep It Off." Mr. Maroney read these representations prior to and at the time of purchase, and understood them as representations and warranties that the Product was, in fact, effective for weight loss and "Clinically Proven" to help consumers "Lose Weight & Keep It Off." He relied on these representations and warranties in deciding to purchase Glanbia's SlimFast MCT product, and these representations and warranties were part of the basis of the bargain in that he would not have purchased Glanbia's SlimFast MCT product if he had known that it was not, in fact, effective for weight loss and not "Clinically Proven" to help consumers "Lose Weight & Keep It Off."

22. Ultimately, Glanbia's SlimFast MCT product was ineffective for weight loss. Moreover, the Product was worthless (and certainly worth less than its Misrepresentations suggested) because it is not, in fact, "Clinically Proven" to help consumers "Lose Weight & Keep It Off." On the contrary, SlimFast MCT may actually be dangerous, let alone ineffective, as the coconut oil contained within it can increase levels of low-density lipoprotein, which is a leading cause of atherosclerosis.

23. Defendant Glanbia Performance Nutrition Inc. d/b/a SlimFast is a Florida corporation with its principal place of business at 3500 Lacey Road, Suite 1200, Downer Grove, Illinois 60515. Glanbia is a wholly owned subsidiary of foreign corporation Glanbia plc. Glanbia is engaged in the manufacturing, processing, packaging, and distribution of SlimFast MCT. Glanbia sells SlimFast MCT throughout New York and the entire United States.

JURISDICTION AND VENUE

24. This Court has subject matter jurisdiction pursuant to 28 U.S.C. § 1332(d)(2)(A) because this case is a class action where the aggregate claims of all members of the proposed class are in excess of $5,000,000.00, exclusive of interest and costs, and Plaintiff, as well as most members of the proposed class, are citizens of states different from Defendant. This Court also has supplemental jurisdiction over state law claims pursuant to 28 U.S.C. § 1367.

25. Pursuant to 28 U.S.C. § 1391, this Court is the proper venue for this action because Plaintiff is a citizen of New York and resides in this District, and because Plaintiff purchased SlimFast MCT in this District. Moreover, Defendant distributed, advertised, and sold SlimFast MCT, which is the subject of the present complaint, in this District.

CLASS REPRESENTATION ALLEGATIONS

26. Plaintiff seeks to represent a class defined as all persons in the United States who purchased Glanbia's SlimFast MCT product (the "Nationwide Class"). Excluded from the Nationwide Class are persons who made such purchase for purpose of resale, Defendant, Defendant's officers, directors, agents, trustees, parents, children, corporations, trusts, representatives, employees, principals, servants, partners, joint venturers, or entities controlled by Defendant, and their heirs, successors, assigns, or other persons or entities related to or affiliated

with Defendant and/or Defendant's officers and/or directors, the judge assigned to this action, and any member of the judge's immediate family.

27. Plaintiff also seeks to represent a subclass of all persons who purchased Glanbia's SlimFast MCT product in New York (the "New York Subclass").

28. Members of the Nationwide Class and New York Subclass are so numerous that their individual joinder herein is impracticable. Defendant's annual sales of SlimFast MCT are in the tens of millions of dollars; thus, members of the Nationwide Class number in the hundreds of thousands and members of the New York Subclass number in the tens of thousands. The precise number of Class members and their identities are unknown to Plaintiff at this time but may be determined through discovery. Class members may be notified of the pendency of this action by mail and/or publication through the distribution records of Defendant and third-party retailers and vendors.

29. Common questions of law and fact exist as to all Class members and predominate over questions affecting only individual Class members. Common legal and factual questions include, but are not limited to: whether SlimFast MCT is actually "Clinically Proven" to help consumers "Lose Weight & Keep It Off."

30. The claims of the named Plaintiff are typical of the claims of the Nationwide Class and New York Subclass in that the named Plaintiff was exposed to and relied on Defendant's false and misleading marketing of SlimFast MCT products and suffered a loss as a result of his SlimFast MCT purchases.

31. Plaintiff is an adequate representative of the Nationwide Class and New York Subclass because his interests do not conflict with the interests of the Class members he seeks to represent, he has retained competent counsel experienced in prosecuting consumer class actions,

and he intends to prosecute this action vigorously. The interests of the Nationwide Class and New York Subclass members will be fairly and adequately protected by Plaintiff and his counsel.

32. The class mechanism is superior to other available means for the fair and efficient adjudication of the claims of the Nationwide Class and New York Subclass members. Each individual Class member may lack the resources to undergo the burden and expense of individual prosecution of the complex and extensive litigation necessary to establish Defendant's liability. Individualized litigation increases the delay and expense to all parties and multiplies the burden on the judicial system presented by the complex legal and factual issues of this case. Individualized litigation also presents a potential for inconsistent or contradictory judgments. In contrast, the class action device presents far fewer management difficulties and provides the benefits of a single adjudication, economy of scale, and comprehensive supervision by a single court on the issue of Defendant's liability. Class treatment of the liability issues will ensure that all claims and claimants are before this Court for consistent adjudication of the liability issues.

COUNT I
(Deceptive Acts Or Practices, New York Gen. Bus. Law § 349)

33. Plaintiff hereby incorporates by reference the allegations contained in all preceding paragraphs of this complaint.

34. Plaintiff brings this claim individually and on behalf of the members of the proposed New York Subclass against Defendant.

35. By the acts and conduct alleged herein, Defendant committed unfair or deceptive acts and practices by misrepresenting that SlimFast MCT was "Clinically Proven" to help consumers "Lose Weight & Keep It Off."

36. The foregoing deceptive acts and practices were directed at consumers.

37. The foregoing deceptive acts and practices are misleading in a material way because they fundamentally misrepresent the characteristics of SlimFast MCT to induce consumers to purchase the same.

38. Plaintiff and members of the New York Subclass were injured because (a) they would not have purchased SlimFast MCT had they known that it was not "Clinically Proven" to help consumers "Lose Weight & Keep It Off," (b) they overpaid for SlimFast MCT because it is sold at a price premium, and (c) SlimFast MCT does not have the characteristics, uses, or benefits promised, namely that it is not effective for weight loss and is not "Clinically Proven" to help consumers "Lose Weight & Keep It Off." As a result, Plaintiff and members of the New York Subclass have been damaged either in the full amount of the purchase price of SlimFast MCT or in the difference in value between SlimFast MCT as warranted and SlimFast MCT as actually sold.

39. On behalf of himself and other members of the Class and New York Subclass, Plaintiff seeks to enjoin the unlawful acts and practices described herein, to recover their actual damages or fifty dollars, whichever is greater, three times actual damages, and reasonable attorneys' fees.

COUNT II
(False Advertising, New York Gen. Bus. Law § 350)

40. Plaintiff hereby incorporates by reference the allegations contained in all preceding paragraphs of this Complaint.

41. Plaintiff brings this claim individually and on behalf of the members of the proposed New York Subclass against Defendant.

42. Based on the foregoing, Defendant has engaged in consumer-oriented conduct that is deceptive or misleading in a material way which constitutes false advertising in violation of Section 350 of the New York General Business Law.

43. The foregoing advertising was directed at consumers and was likely to mislead a reasonable consumer acting reasonably under the circumstances.

44. These misrepresentations have resulted in consumer injury or harm to the public interest.

45. Plaintiff and members of the New York Subclass were injured because (a) they would not have purchased SlimFast MCT had they known that it was not "Clinically Proven" to help consumers "Lose Weight & Keep It Off," (b) they overpaid for SlimFast MCT because it is sold at a price premium, and (c) SlimFast MCT product does not have the characteristics, uses, or benefits promised, namely that it is not effective for weight loss and is not "Clinically Proven" to help consumers "Lose Weight & Keep It Off." As a result, Plaintiff and members of the New York Subclass have been damaged either in the full amount of the purchase price of SlimFast MCT or in the difference in value between SlimFast MCT as warranted and SlimFast MCT as actually sold.

46. On behalf of himself and the members of the New York Subclass, Plaintiff seeks to enjoin the unlawful acts and practices described herein, to recover actual damages or five hundred dollars per violation, whichever is greater, three times actual damages and reasonable attorneys' fees.

COUNT III
(Breach Of Express Warranty)

47. Plaintiff hereby incorporates by reference the allegations contained in all preceding paragraphs of this complaint.

48. Plaintiff brings this claim individually and on behalf of the members of the proposed Nationwide Class and New York Subclass against Defendant.

49. On February 12, 2020, Plaintiff provided Defendant with timely notice of this claim by letter that complied in all respects with U.C.C. § 2-607(3)(a). *See* **Exhibit D**.

50. Defendant, as the designer, manufacturer, marketer, distributor, and/or seller, expressly warranted that SlimFast MCT was "Clinically Proven" to help consumers "Lose Weight & Keep It Off."

51. In fact, SlimFast MCT is not effective for weight loss and is not "Clinically Proven" to help consumers "Lose Weight & Keep It Off." Published literature has revealed that MCT oil does not, in fact, result in weight loss. On the contrary, SlimFast MCT may actually be dangerous, let alone ineffective, as the coconut oil contained within it can increase levels of low-density lipoprotein, which is a leading cause of atherosclerosis.

52. As a direct and proximate cause of Defendant's breach of express warranty, Plaintiff and members of the New York Subclass were injured because (a) they would not have purchased SlimFast MCT had they known that it was not "Clinically Proven" to help consumers "Lose Weight & Keep It Off," (b) they overpaid for SlimFast MCT because it is sold at a price premium, and (c) SlimFast MCT does not have the characteristics, uses, or benefits promised, namely that it is not effective for weight loss and is not "Clinically Proven" to help consumers "Lose Weight & Keep It Off." As a result, Plaintiff and members of the New York Subclass have been damaged either in the full amount of the purchase price of SlimFast MCT or in the difference in value between SlimFast MCT as warranted and SlimFast MCT as actually sold.

COUNT IV
(Breach Of Implied Warranty Of Merchantability)

53. Plaintiff hereby incorporates by reference the allegations contained in all preceding paragraphs of this complaint.

54. Plaintiff brings this claim individually and on behalf of the members of the proposed Nationwide Class and New York Subclass against Defendant.

55. On February 12, 2020, Plaintiff provided Defendant with notice of this claim by letter that complied in all respects with U.C.C. § 2-607(3)(a). *See* **Ex. D**.

56. Defendant breached the warranty implied in the contract for the sale of SlimFast MCT because it could not pass without objection in the trade under the contract description, the goods were not of fair and average quality within the description, and the goods were unfit for their intended and ordinary purpose because SlimFast MCT does not cause weight loss. As a result, Plaintiff and the Nationwide Class and New York Subclass members did not receive the goods as impliedly warranted by Defendant to be merchantable.

57. Plaintiff and the Nationwide Class and New York Subclass members purchased SlimFast MCT in reliance upon Defendant's skill and judgment and the implied warranties of fitness for the purpose.

58. SlimFast MCT was not altered by Plaintiff or the Nationwide Class or New York Subclass members.

59. SlimFast MCT was defective when it left the exclusive control of Defendant.

60. Defendant knew SlimFast MCT would be purchased and used without additional testing by Plaintiff and the Nationwide Class and New York Subclass members.

61. SlimFast MCT was defectively designed and unfit for its intended purpose, and Plaintiff and the Nationwide Class and New York Subclass members did not receive the goods as warranted.

62. As a direct and proximate cause of Defendant's breach of implied warranty, Plaintiff and members of the Nationwide Class and New York Subclass were injured because (a) they would not have purchased SlimFast MCT had they known that it would not actually help them lose weight, (b) they overpaid for SlimFast MCT because it is sold at a price premium, and (c) SlimFast MCT does not have the characteristics, uses, or benefits promised, namely it is not effective for weight loss and is not "Clinically Proven" to help consumers "Lose Weight & Keep It Off." As a result, Plaintiff and members of the New York Subclass have been damaged either in the full amount of the purchase price of SlimFast MCT or in the difference in value between SlimFast MCT as warranted and SlimFast MCT as actually sold.

COUNT V
(Unjust Enrichment)

63. Plaintiff hereby incorporates by reference the allegations contained in all preceding paragraphs of this complaint.

64. Plaintiff brings this claim individually and on behalf of the members of the proposed Nationwide Class and New York Subclass against Defendant.

65. Plaintiff and the Nationwide Class and New York Subclass members conferred benefits on Defendant by purchasing SlimFast MCT.

66. Defendant has been unjustly enriched by retaining the revenues derived from Plaintiff and the Nationwide Class and New York Subclass members' purchases of SlimFast MCT. Retention of those monies under these circumstances is unjust and inequitable because Defendant's sale of SlimFast MCT resulted in purchasers being denied the full benefit of their

purchase because SlimFast MCT is not effective for weight loss and is not "Clinically Proven" to help consumers "Lose Weight & Keep It Off."

67. Because Defendant's retention of the non-gratuitous benefits conferred on them by Plaintiff and the Nationwide Class and New York Subclass members is unjust and inequitable, Defendant must pay restitution to Plaintiff and the Nationwide Class and New York Subclass members for its unjust enrichment, as ordered by the Court.

COUNT VI
(Fraud)

68. Plaintiff hereby incorporates by reference the allegations contained in all preceding paragraphs of this Complaint.

69. Plaintiff brings this claim individually and on behalf of the members of the proposed Nationwide Class and New York Subclass against Defendant.

70. As discussed above, Defendant provided Plaintiff and the Nationwide Class and New York Subclass members with false or misleading material information and failed to disclose material facts about SlimFast MCT, including but not limited to the fact that it is not "Clinically Proven" to help consumers "Lose Weight & Keep It Off." On the contrary, SlimFast MCT may actually be dangerous, let alone ineffective, as the coconut oil contained within it can increase levels of low-density lipoprotein, which is a leading cause of atherosclerosis.

71. The above cited literature was published between 2015 and 2018 and has been widely publicized in the nutrition and fitness community. Despite the fact that such literature has been out for years, and that the American Heart Association has warned against the use of coconut oil, Defendant continues to sell SlimFast MCT to unsuspecting customers. In short, Defendant continues to sell a product that cannot do what it claims to do based on scientific research which has been widely available for over several years. On the contrary, the research

suggests that SlimFast MCT may actually be dangerous, let alone ineffective, as the coconut oil contained within it can increase levels of low-density lipoprotein, which is a leading cause of atherosclerosis.

72. The misrepresentations and omissions made by Defendant, upon which Plaintiff and Nationwide Class and New York Subclass members reasonably and justifiably relied, were intended to induce and actually induced Plaintiff and Class and New York Subclass members to purchase the SlimFast MCT.

73. The fraudulent actions of Defendant caused damage to Plaintiff and the Nationwide Class and New York Subclass members, who are entitled to damages and other legal and equitable relief as a result.

RELIEF DEMANDED

WHEREFORE, Plaintiff, individually and on behalf of all others similarly situated, seeks judgment against Defendant, as follows:

A. For an order certifying the Nationwide Class and the New York Subclass under Rule 23 of the Federal Rules of Civil Procedure and naming Plaintiff as representative of the Nationwide Class and New York Subclass and Plaintiff's attorneys as Class Counsel to represent the Nationwide Class and New York Subclass members;

B. For an order declaring that the Defendant's conduct violates the statutes referenced herein;

C. For an order finding in favor of Plaintiff, the Nationwide Class, and the New York Subclass on all counts asserted herein;

D. For compensatory, punitive, and statutory damages in amounts to be determined by the Court and/or jury;

E. For prejudgment interest on all amounts awarded;

F. For an order of restitution and all other forms of equitable monetary relief;

G. For injunctive relief as pleaded or as the Court may deem proper; and

H. For an order awarding Plaintiff and the Nationwide Class and New York Subclass their reasonable attorneys' fees and expenses and costs of suit.

JURY DEMAND

Pursuant to Federal Rule of Civil Procedure 38(b), Plaintiff demands a trial by jury of any and all issues in this action so triable as of right.

Dated: April 3, 2020

Respectfully submitted,

BURSOR & FISHER, P.A.

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EXHIBIT A

BMJ Open

Randomised trial of coconut oil, olive oil or butter on blood lipids and other cardiovascular risk factors in healthy men and women

Kay-Tee Khaw,[1] Stephen J Sharp,[2] Leila Finikarides,[3,4] Islam Afzal,[5] Marleen Lentjes,[1] Robert Luben,[1] Nita G Forouhi[2]

To cite: Khaw K-T, Sharp SJ, Finikarides L, *et al*. Randomised trial of coconut oil, olive oil or butter on blood lipids and other cardiovascular risk factors in healthy men and women. *BMJ Open* 2018;**8**:e020167. doi:10.1136/bmjopen-2017-020167

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ABSTRACT

Introduction High dietary saturated fat intake is associated with higher blood concentrations of low-density lipoprotein cholesterol (LDL-C), an established risk factor for coronary heart disease. However, there is increasing interest in whether various dietary oils or fats with different fatty acid profiles such as extra virgin coconut oil may have different metabolic effects but trials have reported inconsistent results. We aimed to compare changes in blood lipid profile, weight, fat distribution and metabolic markers after four weeks consumption of 50 g daily of one of three different dietary fats, extra virgin coconut oil, butter or extra virgin olive oil, in healthy men and women in the general population.

Design Randomised clinical trial conducted over June and July 2017.

Setting General community in Cambridgeshire, UK.

Participants Volunteer adults were recruited by the British Broadcasting Corporation through their websites. Eligibility criteria were men and women aged 50–75 years, with no known history of cancer, cardiovascular disease or diabetes, not on lipid lowering medication, no contraindications to a high-fat diet and willingness to be randomised to consume one of the three dietary fats for 4 weeks. Of 160 individuals initially expressing an interest and assessed for eligibility, 96 were randomised to one of three interventions; 2 individuals subsequently withdrew and 94 men and women attended a baseline assessment. Their mean age was 60 years, 67% were women and 98% were European Caucasian. Of these, 91 men and women attended a follow-up assessment 4 weeks later.

Intervention Participants were randomised to extra virgin coconut oil, extra virgin olive oil or unsalted butter and asked to consume 50 g daily of one of these fats for 4 weeks, which they could incorporate into their usual diet or consume as a supplement.

Main outcomes and measures The primary outcome was change in serum LDL-C; secondary outcomes were change in total and high-density lipoprotein cholesterol (TC and HDL-C), TC/HDL-C ratio and non-HDL-C; change in weight, body mass index (BMI), waist circumference, per cent body fat, systolic and diastolic blood pressure, fasting plasma glucose and C reactive protein.

Results LDL-C concentrations were significantly increased on butter compared with coconut oil (+0.42, 95% CI 0.19 to 0.65 mmol/L, P<0.0001) and with olive oil (+0.38, 95% CI 0.16 to 0.60 mmol/L, P<0.0001), with no differences in change of LDL-C in coconut oil compared with olive oil (−0.04, 95% CI −0.27 to 0.19 mmol/L, P=0.74). Coconut oil significantly increased HDL-C compared with butter (+0.18, 95% CI 0.06 to 0.30 mmol/L) or olive oil (+0.16, 95% CI 0.03 to 0.28 mmol/L). Butter significantly increased TC/HDL-C ratio and non-HDL-C compared with coconut oil but coconut oil did not significantly differ from olive oil for TC/HDL-C and non-HDL-C. There were no significant differences in changes in weight, BMI, central adiposity, fasting blood glucose, systolic or diastolic blood pressure among any of the three intervention groups.

Conclusions and relevance Two different dietary fats (butter and coconut oil) which are predominantly saturated fats, appear to have different effects on blood lipids compared with olive oil, a predominantly monounsaturated fat with coconut oil more comparable to olive oil with respect to LDL-C. The effects of different dietary fats on lipid profiles, metabolic markers and health outcomes may vary not just according to the general classification of their main component fatty acids as saturated or unsaturated but possibly according to different profiles in individual fatty acids, processing methods as well as the foods in which they are consumed or dietary patterns. These findings do not alter current dietary recommendations to reduce saturated fat intake in general but highlight the need for further elucidation of the more nuanced relationships between different dietary fats and health.

Trial registration number NCT03105947; Results.

Strengths and limitations of this study

- The randomised trial design comparing three dietary fat interventions minimised confounding and bias.
- There was good compliance and participants were from the general community in a 'real life' setting.
- Objective measures of outcome—blood biochemistry and anthropometry—were used, minimising bias.
- Participants were not blinded as to the intervention, and the intervention was relatively short term over 4 weeks.

INTRODUCTION

This trial was conducted in the context of debate over longstanding dietary recommendations to reduce dietary fat intake for health. The Women's Health Initiative reported no differences in cardiovascular disease in women randomised to low fat and usual diets over 8 years[1] while an intervention comparing a low-fat diet with a Mediterranean diet with extra virgin olive oil or nuts (PREDIMED) reported approximately 30% lower cardiovascular events in both Mediterranean diet arms after 4.8 years[2]; meta-analyses of observational studies and trials report inconsistent findings in the relationship between dietary saturated fatty acids and cardiovascular disease[3 4] and the relationships of dairy fats including milk and butter with cardiovascular disease also being debated.[5–7] Part of the debate relates to the increasing evidence that different individual fatty acids, such as the odd chain or even chain saturated fatty acids, or short, medium and long chain saturated fatty acids, may have different metabolic pathways and subsequent potential health effects as well as the understanding that diet is more complex than individual nutrients or generic biochemical nutrient groups and that contextual factors such as foods and dietary patterns are important. The 2015–2020 US dietary guidelines[8] now focus on foods and dietary patterns and while they recommend limiting saturated and trans fats, they no longer explicitly recommend limiting total fat. In this context therefore, there is renewed interest in the health effects of different fats and oils.

Extra virgin coconut oil has recently been promoted as a healthy oil. Though high in saturated fat, the main saturated fatty acid, lauric acid (c12:0), has been suggested to have different metabolic and hence health effects compared with other saturated fatty acids such as palmitic acid (c16:0), predominant in butter, palm oil and animal fat. In particular, it has been suggested that coconut oil does not raise total cholesterol (TC) or low-density lipoprotein cholesterol (LDL-C) as much as butter. A recent review on coconut oil and cardiovascular risk factors in humans concluded that the evidence of an association between coconut oil consumption and blood lipids or cardiovascular risk was mostly poor quality.[9] While some small studies have been reported comparing coconut oil and butter, these have been small[10 11] and none conducted in the UK where overall dietary patterns are different from Asia, USA or New Zealand where most trials have been conducted. The 2017 American Heart Association Presidential advisory on dietary fats and cardiovascular disease highlighted the paucity of evidence over the long-term health effects of saturated fats such as coconut oil and reinforced strongly recommendations to lower dietary saturated fat and replacement with unsaturated fat to lower LDL-C and prevent cardiovascular disease.[12] In particular, they stated 'because coconut oil increases LDL-C, a cause of cardiovascular disease and has no known off setting favourable effects, we advise against the use of coconut oil'.[12]

Though the PREDIMED study reported lower cardiovascular disease events in those randomised to extra virgin olive oil or added nuts,[2] this trial reported no overall effects on LDL-C or TC for those on olive oil compared with the low-fat diet,[13] results consistent with a review of intervention trials of high phenolic olive oil.[14]

We therefore aimed to examine whether in free living healthy men and women in the UK, we could observe differences in blood lipids after 1 month's consumption of 50 g daily of one of three different fats within the context of their usual diet. Although this was a short-term trial that did not address cardiovascular disease events, blood lipids are a well established risk factor for coronary heart disease and the aim was to compare directly the effects of three different fats, extra virgin coconut oil, butter (both predominantly saturated fats) with extra virgin olive oil (monounsaturated fat) on blood lipid profiles and metabolic measures, in a pragmatic trial using amounts feasible in daily diets.

METHODS

Study population

Participants were volunteers living in the general community predominantly in the Cambridgeshire area, recruited through British Broadcasting Corporation (BBC) advertising in May and June 2017. Eligible participants were men or women aged between 50 and 75 years who did not have a known medical history of heart disease, stroke, cancer or diabetes and who were not taking medication for lowering blood lipids such as statins. They had to be willing to be randomised to consume 50 g daily of one of the designated fats for four weeks and not have any contraindications to eating a high-fat diet such as gall bladder or bowel problems. Of 160 individuals expressing an interest, 96 were eligible and randomised to the intervention, 2 withdrew prior to the start of the study and 94 attended a baseline assessment.

Allocation to intervention

Participants were assigned a unique study identification number (ID). These ID numbers were randomised by computer generated allocation conducted by an independent statistician separately in men and women, into one of three parallel intervention arms approximately equal in size: extra virgin coconut oil, butter or extra virgin olive oil.

Intervention

Participants attending the baseline assessment, at the end of their appointment, received 1 month's supply of one of the three different dietary fats to which they had been randomly allocated: extra virgin coconut oil or butter or extra virgin olive oil. The BBC study organiser was given an ID list with the random allocation to the fats/oils and was responsible for giving each participant their supply of fat/oils. They were asked to eat 50 g of these fats daily for 4 weeks and given measuring cups for the

50 mL fat and oils: butter was prepacked in 20 g and 30 g portions. They were asked to continue with their usual diet and either incorporate the fat or oil into their daily diet to substitute for other fats or oils or they could eat these fats as a supplement. They also had information sheets with suggestions for how the fats could be consumed including recipes. The fats selected were standard products available from supermarkets bought from suppliers; organic extra virgin coconut oil, organic unfiltered extra virgin olive oil and organic unsalted butter. Samples of the oils/fats used in the trial were sent to a reference laboratory: the West Yorkshire Analytic Services, a UKAS accredited testing service for food composition.

Assessments

Participants attended two assessments at a community centre in Cambridge: one at baseline before the start of the intervention in June 2017 and one at the end of 4 weeks in July 2017. Prior to their initial assessment, they were asked to fill in a short questionnaire about their health and lifestyle including physical activity and diet as well as complete an online 24 hours dietary assessment questionnaire with automated nutrient intake estimation, developed in Oxford, the DietWebQ.[15] All assessments were conducted between 08:00 and 12:30 hours. Participants were all fasted for a minimum of 4 hours prior to attending the assessment; the majority were fasted overnight. They had height and waist circumference measured to a standardised protocol in light clothing without shoes and blood pressure measured using an automated OMRON device after being seated resting for 5 min. The mean of two readings for blood pressure, height and waist was used for analysis. Weight and per cent body fat were measured using a Tanita body composition monitor. All measurements were conducted by two trained observers unaware of allocation to the oils/fats. Participants gave a 20 mL blood sample which was stored in a 4°C refrigerator and then sent to the laboratory by courier for same day sample processing and storage for later analysis.

After 4 weeks at the end of the intervention, they attended again for a follow-up assessment where the same measurements of height, waist circumference, blood pressure, weight and per cent body fat were conducted, and another fasting 20 mL blood sample taken. Measurements were recorded on new forms and observers and participants did not have access to the measurements taken at the baseline visit. Just prior to this visit, participants were asked to fill in again the online 24-hour DietWebQ. Participants also filled in short questionnaire about their experiences on the intervention fats. This included a question about their overall experience of consuming the assigned oil/fat in the study where they were asked on average, over the past 4 weeks whether they felt mostly the same as usual, mostly felt better than usual or mostly felt worse than usual with an open-ended section for comments including side effects and overall compliance with consuming the fats which they were asked to self-rate between 0% and 100%. They were also asked whether they changed their type, level or frequency of physical activity in the past month since being in the study and had three options, no overall change in activity, increase in activity or decrease in activity.

Blood samples were identified only by a study ID number and were processed using standard protocols and assayed in two batches at the end of the baseline and follow-up assessments in the Core Biochemical Assay Laboratory (CBAL) Cambridge University Hospitals which has UKAS Clinical Pathology Accreditation; blood samples from individuals on different interventions were thus all assayed in the same batch. The laboratory assays were conducted in a blinded fashion without any indication of the allocated intervention. Cholesterol (TC) and triglycerides were measured using enzymatic assays,[16 17] high-density lipoprotein cholesterol (HDL-C) was measured using a homogenous accelerator selective detergent assay automated on the Siemens Dimension RxL analyser and LDL-C was calculated from the triglyceride, HDL and cholesterol concentrations as described in the Friedewald formula [LDL = Cholesterol - HDL – (Triglycerides/2.2)].[18] Total to HDL-C ratio was computed, and non-HDL-C was computed as TC minus HDL-C.

Plasma glucose was measured using the hexokinase-glucose-6-phosphate dehydrogenase method, and high-sensitivity human C reactive protein was assayed using an automated colourimetric immunoassay: Siemens Dimension CCRP *Cardio*Phase high-sensitivity CRP.

Trial outcomes

The trial was registered in April 2017 with clinical trials registration: NCT03105947. The primary outcome of the trial was change in LDL-C from baseline to follow-up. Secondary outcomes were change in each of the following variables from baseline to follow-up: TC, HDL-C, triglycerides; ratio of TC/HDL-C, non-HDL-C, fasting blood glucose, C reactive protein, weight, body mass index (BMI), body fat %, waist circumference, systolic blood pressure and diastolic blood pressure.

Statistical analysis

The study aimed to recruit a total of 90 participants: 30 individuals per group would provide approximately 80% power to detect a difference in mean within-person change in LDL-C (baseline to follow-up) comparing pairs of randomised groups (butter vs coconut oil and butter vs olive oil) of approximately 0.5 mmol/L, assuming a SD of LDL-C of 1.04 mmol/L[19] and a correlation between baseline and follow-up values of 0.79[20] incorporated using the method described by Borm *et al*.[21] With 2 primary pairwise comparisons, the significance level for each comparison was set to 2.5%.

This magnitude of difference was what can be estimated from metabolic ward studies in which replacement of 10% dietary calories from saturated fat is associated with 0.52 mmol/L cholesterol difference[22] though

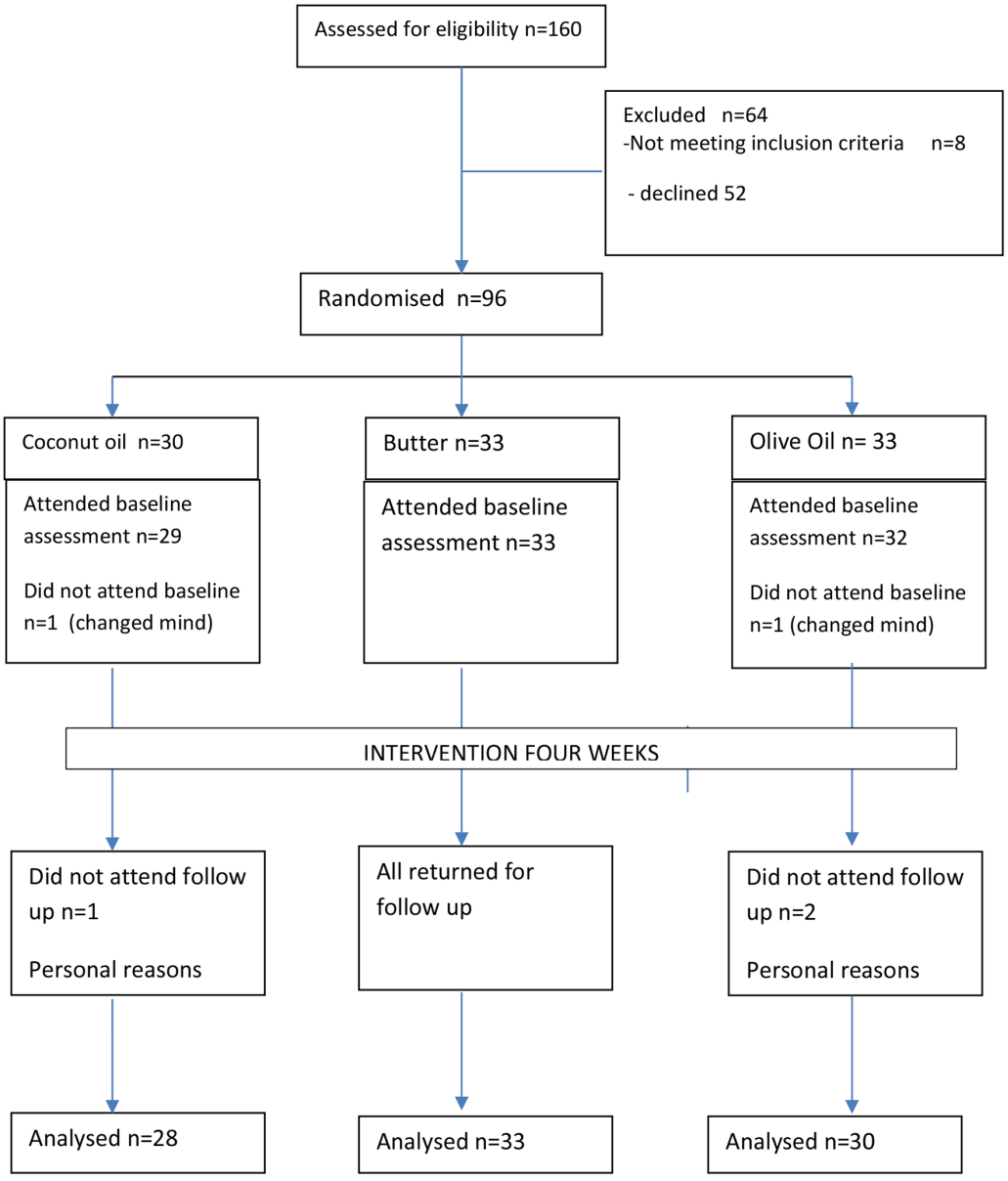

Figure 1 Recruitment and flow diagram (CONSORT) for coconut oil, olive oil or butter trial.

this did not specify the food sources of saturated fats, and a small intervention trial (n=28) comparing butter and coconut oil with sunflower oil.[10]

Baseline characteristics were summarised separately for each randomised group. As recommended by CONSORT, no P values were calculated for this table. The primary analysis used an intention-to-treat (ITT) population, which included all individuals in the group to which they were randomised, regardless of the extent to which they adhered to the intervention. A secondary analysis used a per protocol (PP) population. This was a subset of the ITT population consisting of those individuals who adhered to the intervention. Participants who reported >75% adherence when asked at the follow-up visit were included in the PP population.

For each outcome, a P value was calculated to compare the three randomised groups using a linear regression model, in which change from baseline was the outcome and including a dummy variable for randomised group and the baseline value of the outcome variable as covariates, that is, an analysis of covariance model. Differences between each pair of randomised groups and 95% CIs were also estimated from a similar model.

Patient and public involvement

The BBC originally proposed the idea of a study to examine claims about the health benefits of coconut oil in response to public interest; the study would be part of their 'Trust me, I'm a doctor' series. The study was designed as a randomised trial with participants from the general community in discussion with the BBC.

RESULTS

Figure 1 is the CONSORT diagram for the trial. The recruitment was conducted by the BBC coordinator

through BBC website advertising. From 160 individuals initially expressing an interest and after exclusion criteria, 96 individuals were randomised and invited to a baseline assessment session in June 2017. Two individuals subsequently withdrew and 94 individuals attended the baseline assessment session in June 2017. At the 4-weekfollow-up assessment in July 2017, 91 individuals attended; three individuals did not attend follow-up indicating personal circumstances.

Table 1 shows descriptive characteristics for the participants at the baseline assessment according to the allocation to dietary oils/fats. Two thirds of the participants were women and the mean age overall was 60 years.

Table 2 shows mean changes in the primary and secondary outcomes at the 4-week follow-up within each randomised group and comparisons between each pair of randomised groups. LDL-C concentrations were significantly increased on butter compared with coconut oil (+0.42, 95% CI 0.19 to 0.65 mmol/L, P<0.0001) and olive oil (+0.38 L, 95% CI 0.16 to 0.60 mmol/L, P<0.0001), with no differences in change of LDL-C in coconut oil compared with olive oil (−0.04, 95% CI −0.27 to 0.19 mmol/L, P=0.74). Coconut oil significantly increased HDL-C compared to butter (+0.18 95% CI 0.06 to 0.30 mmol/L) or olive oil (+0.16, 95% CI 0.03 to 0.28 mmol/L).

Butter significantly increased the cholesterol/HDL-C ratio compared with coconut oil (+0.36, 95% CI 0.18 to 0.54) and olive oil (+0.22, 95% CI 0.04 to 0.40) and also increased non-HDL-C compared with coconut oil (+0.39, 95% CI 0.16 to 0.62 mmol/L) and olive oil (+0.39 (95% CI 0.16 to 0.62) but coconut oil did not significantly differ from olive oil for change in cholesterol/HDL-C ratio (−0.14, 95% CI −0.33 to 0.05) or non-HDL-C (0.002, 95% CI −0.23 to 0.24 mmol/L).

Coconut oil also significantly lowered C reactive protein in comparison with olive oil (−0.58, 95% CI −1.12 to −0.04 mg/L) but not compared with butter. There were no significant differences in changes in weight, BMI, central adiposity, fasting blood glucose, systolic or diastolic blood pressure among any of the three intervention groups. For weight, for example, the estimated mean(SD) changes in weight were +0.27 (0.77) kg, 0.04 (1.00) kg and −0.04 (0.84) kg for coconut oil, butter and olive oil, respectively. Adjusting for age, sex and body mass index did not materially alter the results (online supplementary table 1).

Figure 2 shows the difference in the primary outcome (LDL-C) between each pair of randomised groups in the 91 individuals who attended baseline and follow-up. Figures 3–5 show the differences in secondary outcomes comparing butter versus coconut oil, coconut oil versus olive oil and butter versus olive oil, respectively. For comparability, the differences are reported in units of baseline SD for the different outcomes in figures 3–5.

Self-reported compliance was high: 87% of participants reported more than 75% compliance with the intervention over the 4 weeks which was similar among the groups (86% coconut oil, 88% butter and 85% olive oil). Secondary analyses on the 82 participants reporting more than 75% compliance showed similar results (not shown). Reported experience consuming the fats was similar between groups: 57%, 66% and 60% reported feeling no different, 18%, 6% and 13% reported feeling better and 25%, 27% and 23% reported feeling worse in the coconut oil, butter and olive oil groups, respectively. Comparison of dietary intake using the 24-hour DietWebQ showed similar levels of dietary intake across intervention groups at baseline. Following the intervention, total fat intake increased in all intervention groups but estimates for absolute intakes of carbohydrate, protein and alcohol did not differ between intervention groups (table 3). Most of the participants reported no changes in usual physical activity (79%, 73% and 89% no change; 14%, 15% and 4% increased usual physical activity and 7%, 12% and 7% decreased usual physical activity in the coconut oil, butter and olive oil groups, respectively). In a posthoc exploratory analysis, exclusion of individuals who reported increasing usual physical activity had little effect on significant differences between interventions for LDL-C and HDL-C and did not alter the findings for weight change (online supplementary table 2).

Online supplementary appendix 1 shows the fatty acid composition of the three oils/fats used in the intervention. Coconut oil was 94% saturated fatty acids, of which the main components were lauric acid C12:0 (48%), myristic acid C14:0 (19%) and palmitic acid C16:0 (9%). Butter was 66% saturated fatty acids, of which the main components were palmitic acid C16:0 (28%), stearic acid C18:0 (12%) and myristic acid C14:0 (11%). Olive oil was 19% saturated fatty acids, mainly palmitic acid C16 (15%) with stearic acid C18:0 (3%) and 68% monounsaturates with the main component being oleic acid C18:1n9 (64%). These profiles are very similar to those reported from other studies.[9]

DISCUSSION

In this trial, middle-aged men and women living in the general community were randomly allocated to consume 50 g extra virgin coconut oil or 50 g butter or 50 g extra virgin olive oil for 4 weeks. We observed at the end of the trial significantly different changes in LDL-C and HDL-C concentrations between the three intervention groups; in pairwise comparisons, coconut oil did not significantly raise LDL-C concentrations compared with olive oil while butter significantly raised LDL-C concentrations compared with both coconut oil and olive oil. Coconut oil significantly raised HDL-C concentrations compared with both butter and olive oil. Butter also significantly raised cholesterol/HDL-C ratio and non-HDL-C more than both coconut oil and olive oil but there were no differences between coconut oil and olive oil for changes in cholesterol/HDL-C and non-HDL-C.

There were no significant differences in weight or BMI change, change in central adiposity as measured

Table 1 Descriptive characteristics at baseline assessment of participants in the COB trial according to allocation (intention to treat)

	Coconut oil	Butter	Olive oil
	n=29	n=33	n=32
	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	59.1 (6.1)	61.5 (5.8)	59.1 (6.4)
LDL-cholesterol (mmol/L)	3.5 (0.9)	3.5 (0.9)	3.7 (1.0)
Total cholesterol (mmol/L)	5.9 (1.0)	5.9 (1.0)	6.0 (0.9)
HDL-cholesterol (mmol/L)	2.0 (0.5)	1.9 (0.5)	1.8 (0.5)
Cholesterol/HDL ratio	3.2 (0.9)	3.2 (0.8)	3.5 (1.2)
Non-HDL-cholesterol (mmol/L)	3.9 (1.0)	4.0 (0.9)	4.2 (1.1)
Glucose (mmol/L)	5.3 (0.4)	5.4 (0.5)	5.4 (0.5)
Weight (kg)	73.9 (15.1)	70.8 (11.7)	71.1 (14.5)
Waist (cm)	85.4 (11.9)	83.7 (8.1)	86.2 (11.5)
Body fat (%)	29.7 (10.2)	29.2 (9.0)	31.5 (9.6)
Body mass index (kg/m^2)	25.5 (4.5)	24.8 (3.5)	25.0 (4.5)
Systolic blood pressure (mm Hg)	131.4 (18.8)	136.5 (18.8)	133.1 (16.5)
Diastolic blood pressure (mm Hg)	79.8 (9.3)	81.0 (12.0)	78.1 (6.7)
DietWebQ intake/day			
Total energy (MJ)	9.00 (3.70)	8.23 (2.17)	9.51 (3.5)
Protein % energy	14.8 (4.4)	16.0 (3.7)	15.7 (3.0)
Carbohydrate % energy	43.6 (8.9)	41.4 (8.7)	42.7 (11.7)
Total fat % energy	37.3 (7.3)	36.7 (8.7)	36.4 (10.3)
Saturated fat % energy	14.1 (3.6)	13.3 (4.4)	13.4 (4.9)
Alcohol % energy	4.2 (5.4)	5.9 (7.5)	5.1 (6.1)
Hours of walking in past week	8.9 (9.5)	10.9 (12.3)	10.1 (8.7)
Hours of cycling in past week	1.8 (2.6)	2.0 (2.5)	2.7 (5.5)
Hours of other physical exercise in past week	3.4 (3.4)	2.3 (4.0)	1.8 (2.6)
	n=29	n=33	n=32
	Median (IQR)	Median (IQR)	Median (IQR)
Triglycerides (mmol/L)	0.89 (0.74 to 1.10)	0.92 (0.70 to 1.20)	0.94 (0.79 to 1.31)
C reactive protein (mg/L)	1.04 (0.47 to 2.15)	1.08 (0.64 to 2.13)	1.13 (0.58 to 2.67)
	% (N)	% (N)	% (N)
Sex			
Men	37.9 (11)	33.3 (11)	28.1 (9)
Women	62.1 (18)	66.7 (22)	71.9 (23)
Ethnicity			
White	96.6 (28)	97.0 (32)	93.8 (30)
Non-white	3.4 (1)	3.0 (1)	3.1 (1)
Smoking status			
Never	58.6 (17)	66.7 (22)	68.8 (22)
Former	34.5 (10)	33.3 (11)	25.0 (8)
Current	6.9 (2)	0.0 (0)	6.3 (2)
Alcohol consumption in past year			
Never or once per month	20.7 (6)	30.3 (10)	28.1 (9)
1–4 times per week	72.4 (21)	48.5 (16)	59.4 (19)
Almost every day or every day	6.9 (2)	21.2 (7)	12.5 (4)
Highest level of education			
School to age 16	13.8 (4)	12.1 (4)	15.6 (5)
School to age 18	27.6 (8)	9.1 (3)	9.4 (3)
University	58.6 (17)	78.8 (26)	75.0 (24)
Currently in paid job			

Continued

Table 1 Continued

	% (N)	% (N)	% (N)
No	20.7 (6)	45.5 (15)	25.0 (8)
Yes	75.9 (22)	54.5 (18)	75.0 (24)

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

by waist circumference or per cent body fat. There were also no significant differences in change in fasting glucose or systolic and diastolic blood pressure among the three different fat interventions. In pairwise comparison, coconut oil significantly lowered C reactive protein compared to olive oil but there were no significant differences between coconut oil and butter for C reactive protein.

The results were somewhat surprising for a number of reasons. Coconut oil is predominantly (approximately 90%) saturated fat which is generally held to have an adverse effect on blood lipids by increasing blood LDL-C concentrations. However, the saturated fatty acid profiles of different dietary fats vary substantially; coconut oil is predominantly (around 48%) lauric acid (12:0) compared with butter (66% saturated fat) which is about 40% palmitic (16:0) and stearic (18:0) acids, leading to suggestions that coconut oil may not have the same health effects as other foods high in saturated fat.[9] Nevertheless, though reviews on coconut oil and cardiovascular disease risk factors have concluded that the evidence of an association between coconut oil consumption and blood lipids or cardiovascular risk was mostly poor quality,[9] trials have generally reported that coconut oil consumption raises LDL-C in comparison to polyunsaturated oil such as safflower oil, though not as much in comparison to butter.[10 11]

Based on three randomised crossover trials of good scientific quality, one trial reported butter increased LDL-C more than coconut oil which raised LDL-C more compared with safflower oil[10]; a second reported that coconut oil raised LDL-C more than beef fat which raised LDL-C more than safflower oil[23] and a third reported that coconut oil raised LDL-C more than palm oil which raised LDL-C more than olive oil.[24] The current study observed that butter raised LDL-C more than coconut oil but that coconut oil did not differ from olive oil. Two studies showed higher HDL-C with coconut oil compared with other fats whether beef fat, safflower oil or olive oil.[23 24] Thus far, the current results are consistent with previous studies indicating that butter raises LDL-C more than coconut oil and also that coconut oil also raises HDL-C. However, the present study is an exception in not finding any increase in LDL-C compared with an unsaturated oil, in this case, olive oil. In this trial, the difference of 0.33 mmol/L in LDL-C on butter compared with olive oil is consistent with previous studies.[25]

This is the largest trial reported to date on coconut oil and lipids apart from a recent study of 200 individuals with established coronary heart disease comparing coconut oil with sunflower oil over 2 years that reported no differences in blood lipids but virtually all the participants were on statin therapy[26] which makes findings difficult to interpret.

Direct comparisons between studies are problematic because of different oils used; we used extra virgin olive oil as a comparison group rather than a polyunsaturated oil such as safflower or sunflower oil, for feasibility reasons of likely participant compliance with the requirement for 50 g intake daily. The PREDIMED study reported no significant difference in change in LDL-C or TC but significant lowering of the cholesterol/HDL-C ratio in the Mediterranean diet supplemented with extra virgin olive oil compared with a low-fat diet.[2 13] A recent review reported that high phenolic olive oil does not modify the lipid profile compared with its low phenolic counterpart[14] though other studies have reported that extra virgin olive oil decreases LDL-C directly measured as concentrations of apoB-100 and the total number of LDL particles as assessed by NMR spectroscopy.[27 28] We therefore expected coconut oil would raise LDL-C compared with olive oil, but in the current study, we observed no evidence of an overall average increase in LDL-C in individuals allocated either to the coconut oil or olive oil intervention.

Lack of compliance with consuming the dietary fat would lead to no differences between groups and hence explain the lack of differences in LDL-C between coconut oil and olive oil groups. However, in this group of volunteers, reported compliance was high and did not differ between groups; in addition, those in the coconut oil group had significantly greater increases in HDL-C compared with those allocated to olive oil or butter, so lack of compliance is unlikely to be an explanation.

The predominant fatty acid in coconut oil, lauric acid (C12:0) as well as myristic acid (C14:0) are medium chain fatty acids that are rapidly absorbed, taken up by the liver and oxidised to increase energy expenditure which is a possible explanation for why coconut oil may have different effects compared with other saturated fats[29]. It is also possible that differences could be attributed to the use of extra virgin preparations of coconut oil rather than standard coconut oil; different methods of preparation such as the chilling method for virgin coconut oil compared with refined, bleached and deodorised coconut oil may influence phenolic compounds and antioxidant activity,[30] thus, processing of oils changes their composition, biological properties and consequent potential metabolic effects. The variations in possible health effects resulting from variations in processing of different fats is well documented in the

Table 2 Mean change in variables between baseline and follow-up after dietary interventions and pairwise comparisons between fats in 91 participants

	Change from baseline				Pairwise comparisons		
	Coconut oil	Butter	Olive Oil	P value Comparison between groups	Coconut oil vs olive oil	Butter vs Coconut oil	Butter vs olive oil
	n=28	n=33	n=30				
	Mean (SD)	Mean (SD)	Mean (SD)		Difference (95% CI)	Difference (95% CI)	Difference (95% CI)
LDL-cholesterol (mmol/L)	−0.09 (0.49)	0.33 (0.48)	−0.06 (0.39)	<0.001	−0.04 (−0.27 to 0.19)	0.42 (0.19 to 0.65)	0.38 (0.16 to 0.60)
Total cholesterol (mmol/L)	0.22 (0.55)	0.42 (0.59)	0.03 (0.43)	0.022	0.19 (−0.08 to 0.46)	0.19 (−0.08 to 0.45)	0.38 (0.11 to 0.64)
HDL-cholesterol (mmol/L)	0.28 (0.29)	0.09 (0.27)	0.10 (0.15)	0.009	0.16 (0.03 to 0.28)	−0.18 (− 0.30 to −0.06)	−0.02 (−0.14 to 0.09)
Triglycerides (mmol/L)	0.07 (0.58)	−0.001 (0.36)	−0.03 (0.27)	0.65	0.10 (−0.12 to 0.32)	−0.08 (−0.29 to 0.13)	0.02 (−0.19 to 0.23)
Cholesterol/HDL ratio	−0.26 (0.36)	0.10 (0.41)	−0.13 (0.32)	<0.001	−0.14 (−0.33 to 0.05)	0.36 (0.18 to 0.54)	0.22 (0.04 to 0.40)
Non HDL-cholesterol (mmol/L)	−0.06 (0.44)	0.33 (0.51)	−0.07 (0.42)	0.001	0.002 (−0.23 to 0.24)	0.39 (0.16 to 0.62)	0.39 (0.16 to 0.62)
Glucose (mmol/L)	−0.05 (0.49)	0.02 (0.48)	−0.06 (0.49)	0.68	0.01 (−0.23 to 0.25)	0.08 (−0.15 to 0.32)	0.09 (−0.14 to 0.33)
C reactive protein (mg/L)	−0.31 (1.09)	−0.04 (0.93)	0.23 (1.40)	0.11	−0.58 (−1.12 to −0.04)	0.29 (−0.24 to 0.82)	−0.29 (−0.80 to 0.23)
Weight (kg)	0.27 (0.77)	0.04 (1.00)	−0.04 (0.84)	0.42	0.30 (−0.16 to 0.76)	−0.22 (-0.67 to 0.23)	0.08 (−0.36 to 0.52)
Waist (cm)	1.29 (3.31)	0.26 (3.43)	0.59 (3.25)	0.52	0.71 (−1.00 to 2.42)	−0.95 (−2.63 to 0.72)	−0.24 (−1.89 to 1.41)
Body fat (%)	0.24 (1.03)	0.34 (1.31)	0.13 (1.30)	0.82	0.09 (−0.54 to 0.73)	0.10 (−0.52 to 0.72)	0.19 (− 0.42 to 0.81)
Body mass index (kg/m^2)	0.09 (0.27)	0.02 (0.35)	−0.01 (0.29)	0.13	0.10 (−0.06 to 0.26)	−0.07 (− 0.22 to 0.09)	0.03 (−0.12 to 0.18)
Systolic blood pressure (mm Hg)	0.18 (11.46)	−3.79 (11.11)	−3.67 (8.23)	0.29	3.91 (−1.22 to 9.04)	−3.22 (−8.26 to 1.82)	0.69 (− 4.26 to 5.64)
Diastolic blood pressure (mm Hg)	−2.02 (5.71)	−1.33 (6.24)	−0.45 (8.48)	0.81	−0.73 (− 3.88 to 2.42)	0.99 (−2.08 to 4.05)	0.26 (−2.78 to 3.30)

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

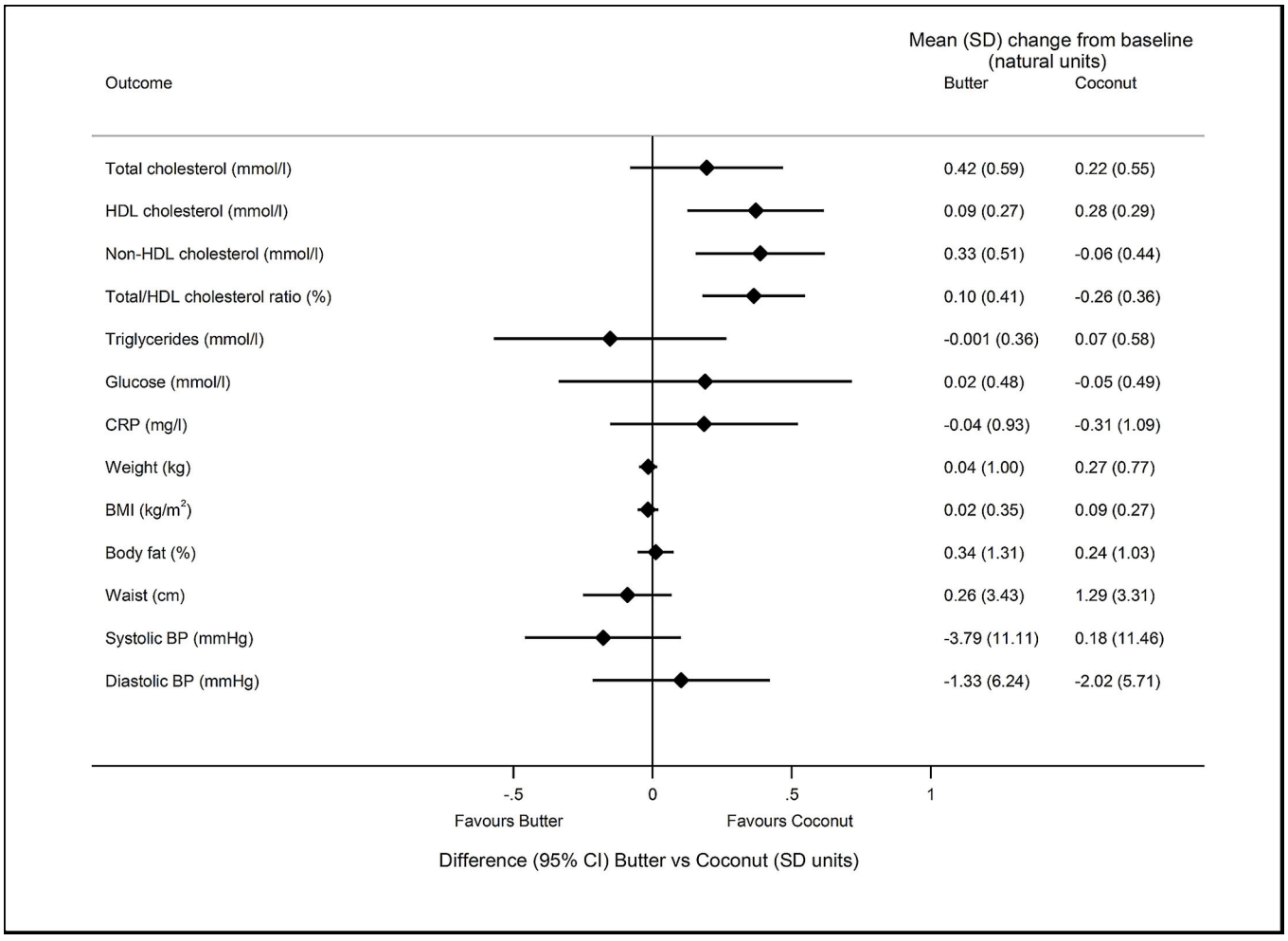

Figure 2 Difference (95% CI) in the primary outcome (LDL cholesterol) between each pair of randomised groups, reported in units of baseline SD. Mean (SD) change from baseline is also presented for each group in mmol/L. COB study, intention-to-treat population, n=91. BMI, body mass index; BP, blood pressure; CRP, C reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

large literature on hydrogenation of polyunsaturated oils to make solid margarines which may increase harmful trans fats.[31]. In this context, it is notable that the major trial (PREDIMED) reporting reduction in cardiovascular risk with a Mediterranean diet used extra virgin olive oil,[2] while other studies which reported null findings with olive oil may not have always specified the product used.[14]

There was no evidence of difference between groups in mean weight, BMI, per cent body fat or central adiposity at the end of this trial; however, these were secondary endpoints for which the trial was not specifically powered. Nevertheless, the estimated 95% CI around mean weight differences at the end for the trial were not large. The participants were asked to consume 50 g of fat or oils daily. They could do this in the context of their usual diet by substituting for their usual fats or by consuming these as a supplement. In practice, most participants reported finding it difficult to substitute the different fats or oils for cooking in their usual diet and usually consumed these as a supplement. These fats if taken in addition to their usual diet would have been approximately 450 additional calories daily, which if consistently taken over 4 weeks might be expected to be nearly 13 000 additional calories resulting in likely weight gain of 1–2 kg. This information was provided in the information sheet with the informed consent for participants. While it is possible that participants may have consciously changed behaviours to maintain body weight such as reducing their other dietary intake because of the additional fat or being more physically active, many participants reported that the high-fat diet resulted in feeling full and eating less.

It is also possible that even though this was a randomised trial, in an unblinded study, participants may have changed behaviours differentially in the different intervention groups resulting in differences in lipids or lack of differences in weight observed rather than being attributed to the dietary fat interventions. The majority of the participants reported no change in usual physical activity though slightly more participants in the coconut oil and butter groups reported increasing usual physical activity (14% and 15%, respectively) compared with 4% in the olive oil group. Nevertheless exclusion of all individuals reporting increased usual physical activity from the analyses did not change the findings. Dietary factors

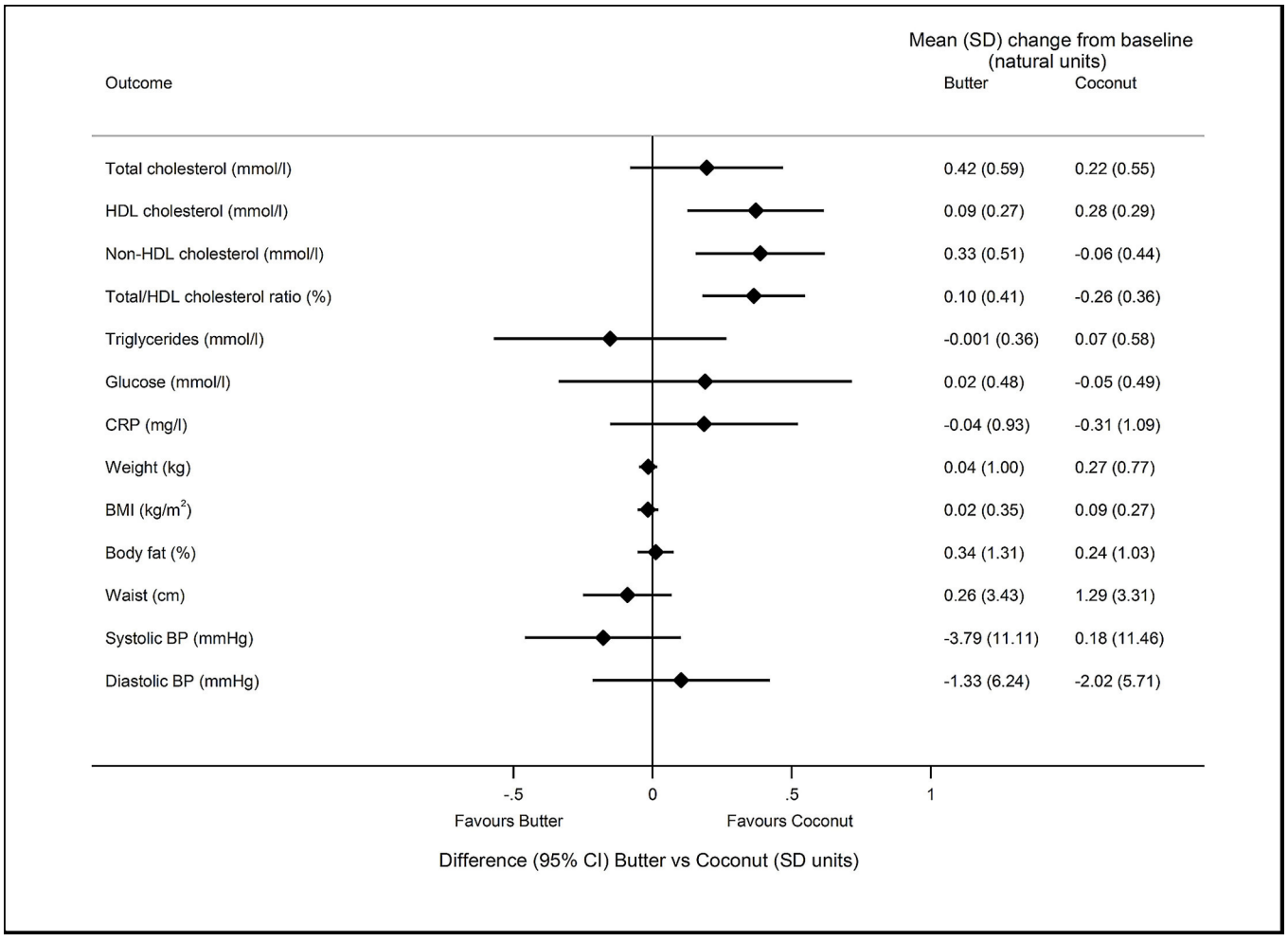

Figure 3 Difference (95% CI) in secondary outcomes comparing butter vs coconut oil groups, reported in units of baseline SD. Mean (SD) change from baseline is also presented for each group in the natural units of the outcome. COB study, intention-to-treat population, n=91. For HDL cholesterol, sign of difference and 95% CI is the opposite of that reported in table 2, on the assumption that higher HDL is better, so the negative estimated difference (butter vs coconut) reported in table 2 is presented on the side of the graph which favours the coconut group. BMI, body mass index; BP, blood pressure; CRP, C reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

apart from fat most likely to influence HDL-C, total alcohol intake or change in alcohol intake did not differ significantly between intervention groups and in fact alcohol intake decreased slightly during the trial which would not explain any increases in HDL-C observed. There is therefore no evidence to suggest that differences in lipids or lack of differences in weight change were likely to be attributed to differential changes in behaviour.

The main strengths of this study are the randomised design with high completion rate (91/94 individuals returned to follow-up) and self-reported dietary compliance (nearly 90% participants with over 75% adherence) over 4 weeks. This is also larger than most trials reported with the exception of the trial in India in individuals with heart disease most of whom were taking statins[26]. The current trial by contrast was conducted in individuals in the general population.

This trial has limitations. It was a short-term trial of 4 weeks intervention, so we are unable to know what would have happened if the intervention had continued for a longer period. Moreover, the current findings only apply to the intermediate metabolic (lipid) risk markers and cannot be extended to findings for clinical endpoints.

It was designed as a pragmatic trial in free living individuals rather than a controlled metabolic ward trial such that individuals were asked only to consume the 50 g of allocated fat or oil daily. As this was a 'real-world' study, we made no attempt to control other aspects of their usual diet in particular, total energy intake. For this reason, our results cannot be taken to reflect what would happen when the only change to a diet is the substitution of one fat with another (eg, replacing butter with coconut oil or replacing butter with olive oil). Individuals may have changed their behaviours in different ways to accommodate this additional fat, whether by modifying other aspects of their diet for instance, increasing foods such as bread and potatoes or salads to eat with the fats or consciously reducing other food intake or changing physical activity patterns to control energy balance. Nevertheless, this trial is more reflective of real-life situations.

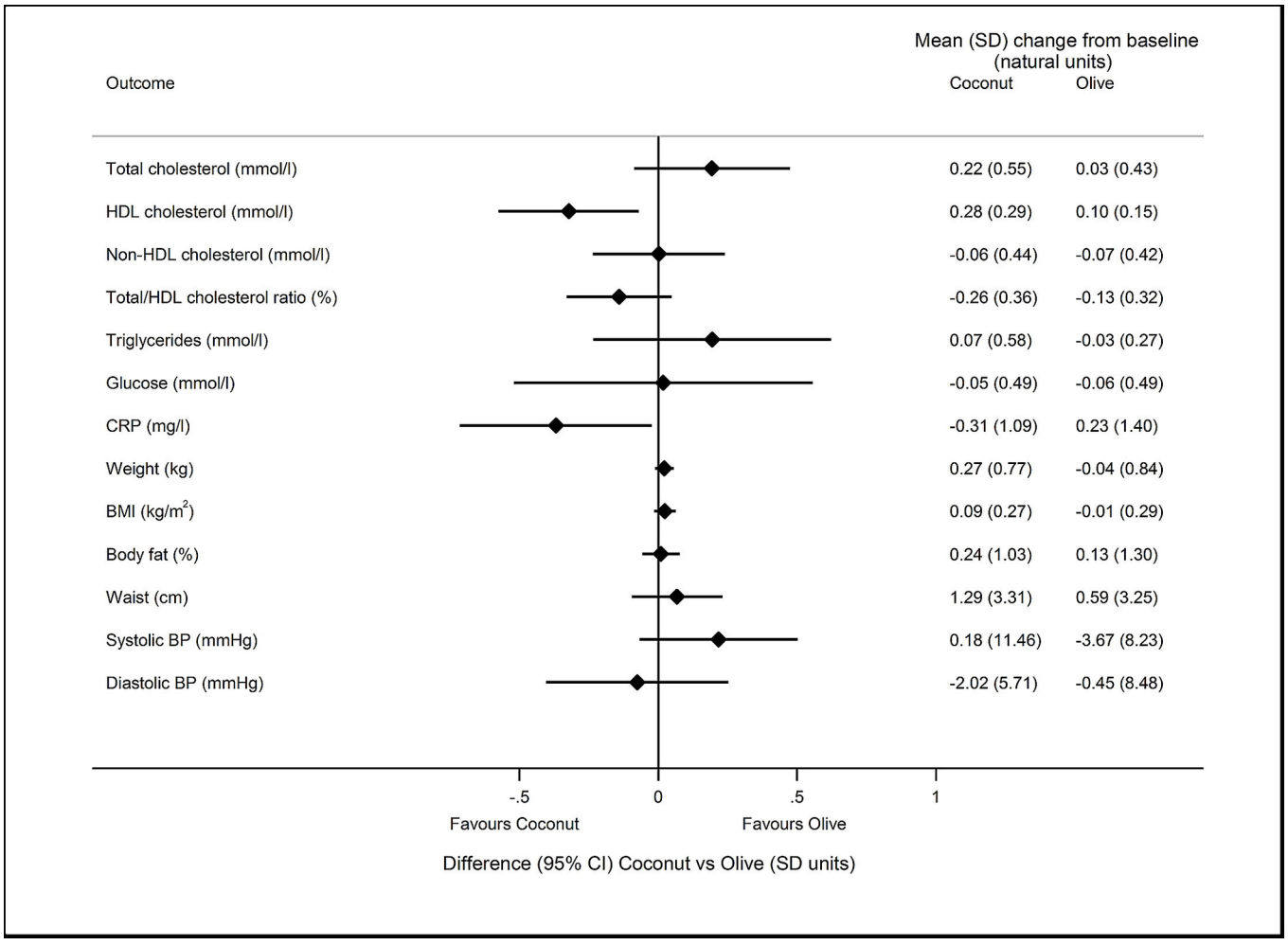

Figure 4 Difference (95% CI) in secondary outcomes comparing coconut oil vs olive oil groups, reported in units of baseline SD. Mean (SD) change from baseline is also presented for each group in the natural units of the outcome. COB study, intention-to-treat population, n=91. For HDL-cholesterol, sign of difference and 95% CI is the opposite of that reported in table 2, on the assumption that higher HDL is better, so the positive estimated difference (coconut vs olive) reported in table 2 is presented on the side of the graph which favours the coconut group. BMI, body mass index; BP, blood pressure; CRP, C reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

While self-reported compliance was high, this was subjective and we did not measure the blood fatty acid profile in participants following the intervention for an objective biomarker of compliance. Nevertheless, we did observe differential changes in blood lipids during the intervention.

The generalisability of the findings to the wider population is also unclear. The volunteers were clearly highly selected to be willing to participate in such a study and also likely to be healthier than the general population, as for ethical reasons we excluded those with known prevalent cardiovascular disease, cancer or diabetes and also those on any lipid lowering medication or other contraindications to a high-fat diet. Nevertheless, it is unlikely that the effect of these dietary fats in this group of individuals recruited from the general population would be biologically different from the general population.

Implications

We focused on LDL-C for the primary endpoint as the causal relationship between LDL-C concentrations and coronary heart disease risk is well established, with about a 15% increase in coronary heart disease risk per 1 mmol/L increase in LDL-C concentrations and reduction of LDL-C cholesterol lowers coronary heart disease risk.[32] Increase in LDL-C concentrations has been the main mechanism through which dietary saturated fat is believed to increase heart disease risk, though other pathways have been postulated. However, it is notable that some Mediterranean diet interventions such as the Lyon heart study (alpha linolenic acid)[33] or PREDIMED (extra virgin olive oil)[2] which have been reported to reduce cardiovascular risk in secondary and primary prevention may have effects through other pathways such as inflammation or endothelial function.[34 35] Whatever the mechanisms, the evidence from prospective studies is consistent and strong that substitution of saturated fats by unsaturated fats is beneficial for cardiovascular risk.[36]

The results of this study indicate that two different dietary fats (coconut oil and butter), which are predominantly saturated fats, appear to have different effects

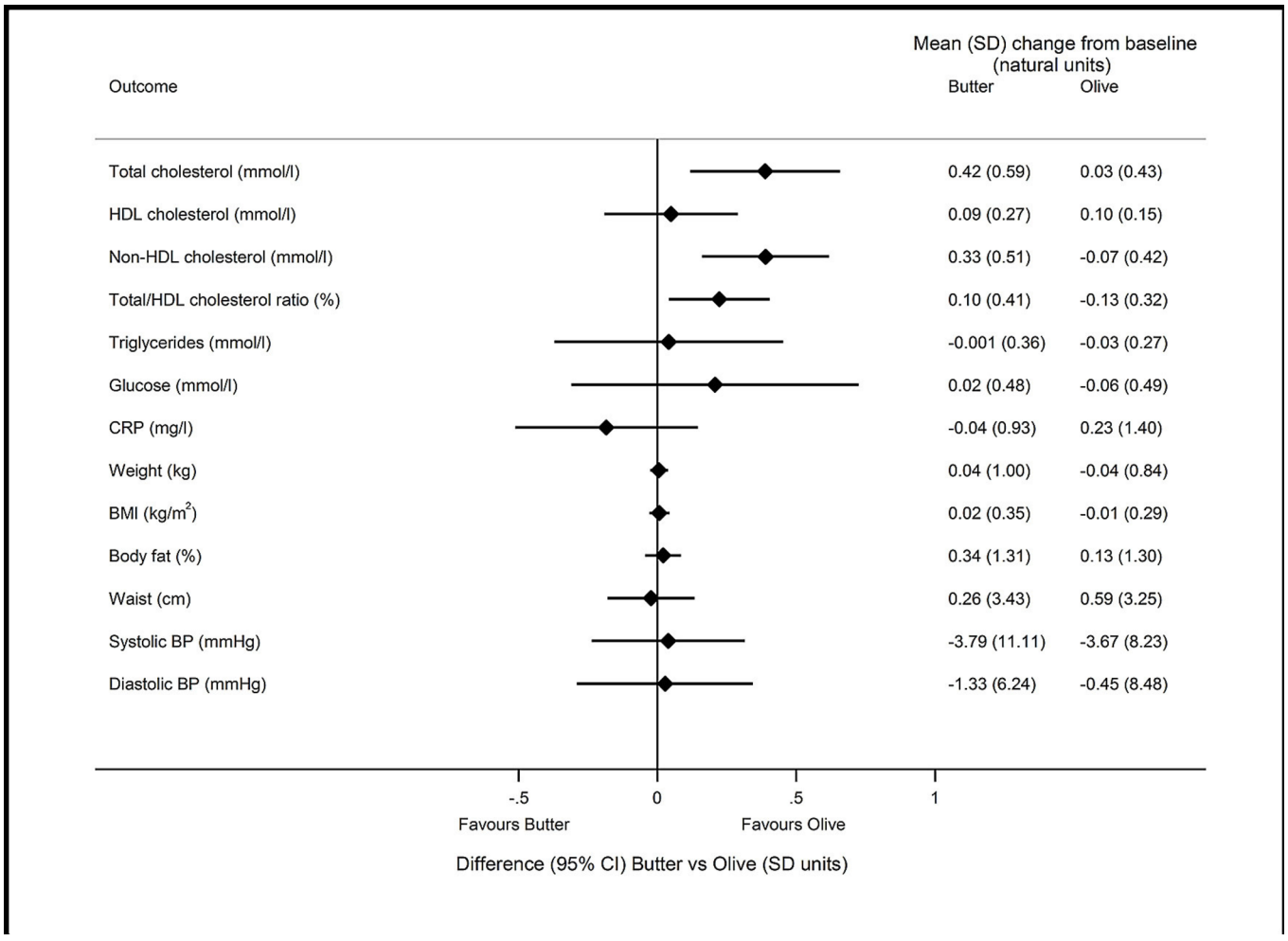

Figure 5 Difference (95% CI) in secondary outcomes comparing butter vs olive oil groups, reported in units of baseline SD. Mean(SD) change from baseline is also presented for each group in the natural units of the outcome. COB study, intention-to-treat population, n=91. For HDL-cholesterol, sign of difference and 95% CI is the opposite of that reported in table 2, on the assumption that higher HDL is better, so the negative estimated difference (butter vs olive) reported in table 2 is presented on the side of the graph which favours the olive group. BMI, body mass index; BP, blood pressure; CRP, C reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

on blood lipids compared with olive oil, a predominantly monounsaturated fat. The effects of different dietary fats on lipid profiles, metabolic markers and health outcomes may vary not just according to the general classification of their main component fatty acids as saturated or unsaturated but possibly according to different profiles in individual fatty acids, processing methods as well as the foods in which they are consumed or dietary patterns. There is increasing evidence that associations of saturated fatty acids with health outcomes may vary according to whether they are odd or even chain saturated fatty acids or their chain length.[37–39] Indeed, while overall the evidence indicates the substitution of dietary saturated fats with polyunsaturated fats is beneficial for coronary heart disease risk[40] heterogeneity in findings from observational studies and trials may reflect different dietary sources of fats.[4 41] As the Joint FAO/WHO 2008 Expert Consultation on Fats and Fatty Acids in Human Nutrition comments:

'There are inherent limitations with the convention of grouping fatty acids based only on number of double bonds…major groups of fatty acids are associated with different health effects…individual fatty acids within each broad classification may have unique biological properties or effects…Intakes of individual fatty acids differ across world depending on predominant food sources of total fats and oils.' The associations with health endpoints may well vary depending on the food sources.

In this trial, extra virgin coconut oil was similar to olive oil and did not raise LDL-C in comparison with butter. The current short-term trial on an intermediate cardiovascular disease risk factor, LDL-C, does not provide evidence to modify existing prudent recommendations to reduce saturated fat in the diet as emphasised in most consensus recommendations[8 12] and dietary guidelines should be based on a range of criteria.[42] However, the findings highlight the need for further elucidation of the more nuanced relationships between different dietary fats and health. There is increasing evidence that to understand

Table 3 Baseline and follow-up dietary intake by allocation to coconut oil, butter or olive oil* estimated using 24-hour DietWebQ

DietWebQ intake/day	Coconut oil	Butter	Olive oil
Baseline prior to start of intervention	n=27	n=33	n=32
Energy (MJ/day)	9.0 (3.7)	8.2 (2.2)	9.5 (3.5)
Total fat (g/day)	94 (47)	81 (26)	98 (50)
Protein (g/day)	74 (29)	75 (19)	87 (34)
Carbohydrate (g/day)	238 (95)	215 (75)	243 (95)
Alcohol (g/day)	16 (22)	17 (23)	18 (22)
At 4 weeks of intervention	n=24	n=32	n=27
Energy (MJ/day)	9.6 (3.2)	8.6 (2.4)	9.6 (3.1)
Total fat (g/day)	127 (47)	94 (37)	138 (38)
Protein (g/day)	71 (25)	77 (29)	78 (31)
Carbohydrate (g/day)	215 (84)	214 (64)	197 (101)
Alcohol (g/day)	9 (15)	13(15)	8 (18)
Change from baseline	n=24	n=32	n=27
Energy (MJ/day)	0.3 (2.9)	0.5 (2.0)	−0.4 (2.8)
Total fat (g/day)	29 (43)	14 (36)	28 (40)
Protein (g/day)	−7 (33)	3 (30)	−12 (26)
Carbohydrate (g/day)	−31 (74)	4 (69)	−55(81)
Alcohol (g/day)	−8 (22)	−5(23)	−11 (27)

*Numbers do not total 94 as not all participants completed the baseline and follow-up DietWebQ.

the relationship between diet and health, we need to go beyond simplistic associations between individual nutrients and health outcomes and examine foods and dietary patterns as a whole. In particular, present day diets with high intakes of processed foods now incorporate many fats and oils such as soya bean oil, palm oil and coconut oil which have not been previously widely used in Western societies and not well studied. The relationships between different dietary fats, particularly some of the now more commonly used fats, and health endpoints such as cardiovascular disease events need to be better established.

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Contributors K-TK had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: KT-K, NGF, LF. Acquisition of data: KT-K, NGF, LF, IA, RL, ML. Analysis and interpretation of the data: KT-K, NGF, LF. Drafting of the manuscript: KT-K. Critical revision of the manuscript for important intellectual content: NGF, SJS, IA, LF, RL, ML. Obtaining funding: KT-K, NGF, LF. Administrative, technical or material support: KT-K, NGF, LF, IA, RL, SJS, ML.

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Disclaimer The lead author and guarantor K-TK affirms that the manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics approval was given for the study by the University of Cambridge Human Biology Research Ethics committee HBREC 2017.05.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data are available. Please contact corresponding author.

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EXHIBIT B

RESEARCH
Review

Effects of Medium-Chain Triglycerides on Weight Loss and Body Composition: A Meta-Analysis of Randomized Controlled Trials

Karen Mumme, PGDipSc; Welma Stonehouse, PhD

ARTICLE INFORMATION

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Obesity
Blood lipids
Body weight

Supplementary materials:
PowerPoint presentation available at www.andjrnl.org

http://dx.doi.org/10.1016/j.jand.2014.10.022

ABSTRACT

Background Medium-chain triglycerides (MCTs) may result in negative energy balance and weight loss through increased energy expenditure and lipid oxidation. However, results from human intervention studies investigating the weight reducing potential of MCTs, have been mixed.

Objective To conduct a systematic review and meta-analysis of randomized controlled trials comparing the effects of MCTs, specifically C8:0 and C10:0, to long-chain triglycerides (LCTs) on weight loss and body composition in adults. Changes in blood lipid levels were secondary outcomes.

Methods Randomized controlled trials >3 weeks' duration conducted in healthy adults were identified searching Web of Knowledge, Discover, PubMed, Scopus, New Zealand Science, and Cochrane CENTRAL until March 2014 with no language restriction. Identified trials were assessed for bias. Mean differences were pooled and analyzed using inverse variance models with fixed effects. Heterogeneity between studies was calculated using I^2 statistic. An I^2>50% or P<0.10 indicated heterogeneity.

Results Thirteen trials (n=749) were identified. Compared with LCTs, MCTs decreased body weight (−0.51 kg [95% CI−0.80 to −0.23 kg]; P<0.001; I^2=35%); waist circumference (−1.46 cm [95% CI −2.04 to −0.87 cm]; P<0.001; I^2=0%), hip circumference (−0.79 cm [95% CI −1.27 to −0.30 cm]; P=0.002; I^2=0%), total body fat (standard mean difference −0.39 [95% CI −0.57 to −0.22]; P<0.001; I^2=0%), total subcutaneous fat (standard mean difference −0.46 [95% CI −0.64 to −0.27]; P<0.001; I^2=20%), and visceral fat (standard mean difference −0.55 [95% CI −0.75 to −0.34]; P<0.001; I^2=0%). No differences were seen in blood lipid levels. Many trials lacked sufficient information for a complete quality assessment, and commercial bias was detected. Although heterogeneity was absent, study designs varied with regard to duration, dose, and control of energy intake.

Conclusions Replacement of LCTs with MCTs in the diet could potentially induce modest reductions in body weight and composition without adversely affecting lipid profiles. However, further research is required by independent research groups using large, well-designed studies to confirm the efficacy of MCT and to determine the dosage needed for the management of a healthy body weight and composition.
J Acad Nutr Diet. 2015;115:249-263.

OBESITY CONTINUES TO BE ONE OF THE BIGGEST global challenges in the 21st century, with at least 2.8 million adults dying each year from conditions resulting from being overweight or obese.[1] In addition, 44% of diabetes burden, 23% of ischemic heart disease burden, and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity.[1] The rising obesity rates can be ascribed to changing dietary patterns and lifestyle that have led to energy-dense diets with reduced physical activity.[1] High-fat diets are often blamed for increased obesity rates; however, fats are diverse and respond differently in vivo.

Medium-chain triglycerides (MCTs) may counteract fat deposition in adipocytes by increasing thermogenesis and satiety.[2] The MCTs contain 8 to 12 carbon atoms and include caprylic acid (C8:0, octanoic acid), capric acid (C10:0, decanoic acid), and lauric acid (C12:0, dodecanoic acid). Foods high in MCTs include coconut oil (58%), palm kernel oil (54%), desiccated coconut (37%), and raw coconut meat (19%) (US Department of Agriculture National Nutrient Database). Average intakes of 1.35 g/day (0.7% of total energy intake) MCTs have been reported in the United States[3] and 0.2 g/day in Japan.[4] MCT is cleaved into glycerol and medium-chain fatty acids in the gut lumen.[5] The medium chain length

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makes a smaller, more soluble molecule compared with a long-chain fatty acid, giving it a preferential absorption and metabolic route in the body.[6] This physicochemical nature of medium-chain fatty acids allows them to pass into the portal vein on route to the liver to be rapidly metabolized via β oxidation with no requirement of reesterification in intestinal cells, incorporation into chylomicrons,[7] or the rate limiting enzyme carnitine acyltransferase for intramitochondrial transport.[5] In comparison, long-chain fatty acids have a slower route, being re-esterified in the small intestine and transported by chylomicrons via the lymphatic and vascular system before being oxidized for energy or stored.[7] Thus, rapid metabolism of MCTs reduces their opportunity of adipose tissue uptake.[8]

Studies in animals and human beings have shown increased energy expenditure and lipid oxidation with MCTs, specifically C8:0 and C10:0, compared with long-chain triglycerides (LCTs).[5,9-16] Increased satiety, resulting in reduced food intake, is another possible benefit from the fast oxidation of MCT through the formation of ketones,[7] although studies in humans have been inconsistent with some showing no effect,[7,9,17,18] whereas others showed increased satiety.[19,20] Replacement of MCT with LCT in the diet, therefore, has the potential to result in negative energy balance and weight loss over the long term.

Several human intervention studies have been conducted investigating the weight-reducing potential of MCT, with mixed results.[21] If weight loss attributes are confirmed for MCT, its incorporation into the diet could have important clinical application for healthy body weight management. MCTs may be included into the diet through the development of food products, such as oils, where proportions of LCT are replaced with MCT for commercial and domestic use or weight loss products (eg, meal replacements). The availability of modern fat technologies enables the production of food products containing higher amounts of MCT that are suitable for cooking and other purposes.

The association between dietary fats and low-density lipoprotein (LDL) cholesterol is well established[22]; therefore, it is important to consider the effects of MCT on blood lipid profiles to ensure no adverse effects. Results from intervention trials with MCT on lipid profiles have been inconsistent, with some reporting unfavorable changes[23-25] and others no change.[24,25]

The primary aim of our study was to conduct a systematic review and meta-analysis of randomized controlled trials (RCTs) comparing the effects of MCT, specifically C8:0 and C10:0, to LCT on weight loss and body composition in healthy adults. Subgroup analyses were conducted to determine whether sex, baseline body weight status, length of intervention, or dosage affected the outcomes. The primary outcome measures were body mass, waist and hip circumference, total body fat, and subcutaneous and visceral fat. Secondary outcomes were blood lipids, including triglycerides (TG), total cholesterol, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol.

METHODS

Eligibility Criteria

We reviewed RCTs, both parallel and crossover, published until March 2014 with no language restrictions. Only studies >3 weeks' duration were reviewed. All studies were peer reviewed and conference abstracts were excluded. In addition, the participants were required to be healthy and older than age 18 years. A body weight measurement was required at baseline and at the end point. Both ad libitum, weight maintaining, and reduced-calorie diets were included, but any exercise intervention studies were excluded. The interventions were to include C8:0 and C10:0 acids with the control containing a longer-chain fatty acid. A formal review protocol was not published.

Literature Search

A systematic search was performed for all studies published before March 31, 2014, through several journal databases, including Web of Knowledge, Discover, PubMed, Scopus, New Zealand Science, and Cochrane CENTRAL. Reference lists of included trials were scanned for further relevant studies. A pilot literature search tested suitability of search words. The final search terms included *medium-chain fatty acid*, *medium-chain triglyceride*; *octanoic*, *decanoic*, *dodecanoic*, *caprylic*, *capric*, *or lauric*; *obesity*, *weight loss*, *overweight*, *body composition*, or *energy expenditure*; and *trial*, *intervention*, or *study*. Results from each database were downloaded into EndNote (version X4.0.2, 2010, Thomson Reuters). Duplicates were removed and abstracts were screened. Where an abstract met the eligibility requirements, the full article was read to ensure inclusion and exclusion criteria were met.

Data Extraction and Quality Assessment

An extraction form was drafted to collect study details on authors; sources of funding; aim, objective, and hypothesis; study design and setting; population; intervention and delivery methods; possible confounders; outcomes; results; conclusions; and assessment of bias risk. The extraction table was piloted on one article independently by the authors and adapted to exclude any sources of discrepancies. Outcome results were collected in Excel (version 14.0.6129.5000, 2010, Microsoft Corp) for further calculation if required. Blood lipid results were converted, where necessary, to milligrams per deciliter from millimoles per liter using a conversion factor of 88.57 for TG and 38.6 for cholesterol.

A bias assessment was performed following the Cochrane risk of bias assessment[26] to assess the validity of studies. Selection, performance, detection, attrition, and reporting bias were reviewed and assessed at the study level. Crossover trials underwent a further assessment of bias risk to consider whether the effects of the first dietary intervention could be reversed during the washout period, whether any carryover effects could bias the second treatment period, whether the treatment order was randomized, and whether a paired statistical analysis was used.[26] In addition, commercial bias was identified where one or more of the authors were employees of the supplier of MCT trial products, there was a conflict of interest, and the control of data and publishing of results were not clearly stated.

Where a study had more than two arms, only relevant arms were selected for comparison and care was taken not to double count participants in the meta-analysis. Where a trial registration number was provided, the registry file was searched to determine reporting bias. Data for each study were extracted independently by one author and verified by the other. Any differences were resolved by discussion.

Statistical Analysis

Data were analyzed using Review Manager (version 5.2.11, 2012, The Nordic Cochrane Centre, The Cochrane Collaboration). A fixed-effects model and inverse variance method was used to calculate the forest plot with a 95% CI. A random effects model was applied in subgroupings to minimize the risk of a false positive result.[26] The fixed-effect estimate gives the best estimate of the intervention effect, whereas the random-effect estimate gives an average intervention effect.[26]

For each outcome, the mean difference of change scores from baseline between intervention groups was used plus the standard deviation (SD). If the SD was not given, it was calculated from the standard error. If the SD or standard error was not available, it was imputed from the mean correlation coefficient for an outcome from other studies in the meta-analysis.[26] For outcomes using differing units of measure that could not be converted (eg, subcutaneous and visceral body fat were measured in kilograms and centimeters^3), the standardized mean difference (SMD) was calculated.

Heterogeneity between studies was calculated using the I^2 statistic. Heterogeneity was identified when $I^2>50\%$ or $P<0.10$.[26] If heterogeneity was identified in any outcome, the reasons were explored or a random effects model was used.[26] If the heterogeneity could not be rectified, the meta-analysis for that particular outcome was not completed.

Where there were 10 or more studies for an outcome, publication bias was calculated using the Egger linear regression method.[27] Subgroup analysis was done a priori on study duration (<12 weeks or ≥12 weeks), sex, and MCT intake (<8%, 8% to 16%, and >16% energy). It was not possible to do a subgroup analysis by baseline body weight status due to the overlapping of groups. Post hoc analysis was done on commercial- vs noncommercial-biased studies. Subgroup differences were assessed using χ^2 test. Sensitivity analysis was done on trials with a high bias (two or more high-risk assessment), trials with imputed data, crossover trials, and trials with high commercial bias. Results are shown as weighted mean difference or SMD with 95% CIs. Statistical significance was set at $P<0.05$.

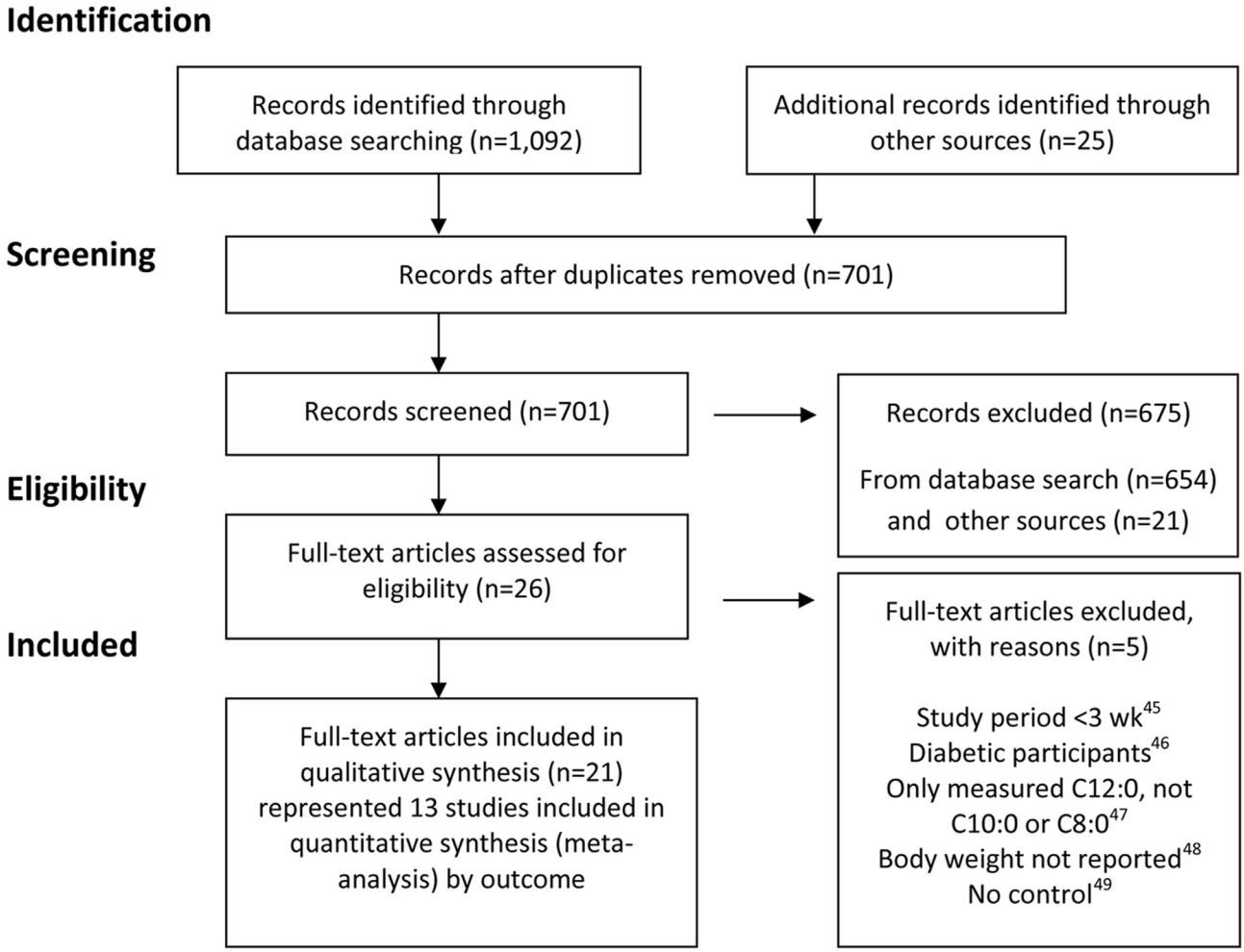

Figure 1. Preferred reporting items for systematic reviews and meta-analyses flow diagram for search of randomized controlled trials used in meta-analysis. Databases searched included Web of Knowledge, Discover, PubMed, Scopus, New Zealand Science, and Cochrane CENTRAL. Other sources of literature included reference lists of full-text articles included in meta-analyses.

Table. Characteristics of randomized controlled trials examining the effect of medium-chain triglycerides on weight, body composition, and blood lipid levels selected for this meta-analysis

Study[a]	Methods and duration	Participants	Diet		Outcomes
			Intervention	Control	
Yost and Eckel 1989[34]	DB[b] P[c] 4-12 wk	16 obese women, aged 29-44 y in Colorado	800 kcal/d via formula containing 47% energy from CHO[d], 22% energy from protein, 6% energy from LCT[e] and 24% energy from MCT[f] (~21 g/d)	800 kcal/d via formula containing 47% energy from CHO, 22% energy from protein, 31% energy from LCT (soy oil)	BW[g]
Temme and colleagues 1997[28]	SB[h] P 6 wk	60 adults, BMI[i] 20-30, in the Netherlands	Margarine and foods, 10% energy from MCT (~24 g/d)	i) Margarine and foods, 10% energy from myristic acid (C14:0) ii) Margarine and foods, 17% energy from oleic acid	BW, TG[j], total cholesterol, HDL[k] cholesterol, LDL[l] cholesterol
Feldheim 2001[36]	C-O[m] 2×4 wk + 2 wk washout	35 women, aged 19-24 y, normal BMI, in Prague	Fat provided, 5% energy from MCT (~12.5 g/d)	Unknown LCT	BW
Krotkiewski 2001[19]	DB P 4 wk	66 obese, perimenopausal women in Sweden	579 kcal/d via Adinax[n] powder; 13% energy from MCT (9 g/d), 21% energy from CHO, 20% energy from protein, mixed with nonfat milk (45% of energy)	579 kcal/d via Adinax powder; 13% energy from LCT, 21% energy from CHO, 20% energy from protein, mixed with nonfat milk (45% of energy)	BW, body fat, TG, total cholesterol
			3 liquid meals per day		
Matsuo and colleagues 2001[30,o]	P 12 wk	13 men aged 18-20 y, normal BMI, in Japan	Liquid formula supplement, 20 g SMLCT[p], <1% energy from MCT	Liquid formula supplement, 20 g soybean oil	BW, total adipose, TG, total cholesterol, HDL cholesterol, LDL cholesterol
			plus other ad libitum food		
Tsuji and colleagues 2001[33,o]	DB P 12 wk	78 adults in Japan	Breakfast bread containing ~4% energy from MCT (10 g/d)	Breakfast bread containing 10 g/d LCT (70% rapeseed, 30% soybean oil)	BW, body composition, TG and total cholesterol
			Pre-packaged lunch, dinners provided plus 250 g fruit and vegetables/day		

(*continued on next page*)

Table. Characteristics of randomized controlled trials examining the effect of medium-chain triglycerides on weight, body composition, and blood lipid levels selected for this meta-analysis (*continued*)

Study[a]	Methods and duration	Participants	Diet		Outcomes
			Intervention	Control	
Kasai and colleagues 2003[4,o]	DB P 12 wk	82 adults, mean BMI=25, in Japan	Breakfast bread containing 14 g MLCT[q], providing 1.7 g MCT, <1% energy Pre-packaged lunch, dinners provided plus 250 g fruit and vegetables/day	Breakfast bread containing 14 g LCT (70% rapeseed, 30% soybean oil)	BW, body composition, TG, total cholesterol, HDL cholesterol, and LDL cholesterol
Nosaka and colleagues 2003[31,o]	DB P 12 wk	64 adults, mean BMI=25, in Japan	14 g margarine/d containing <2% energy from MCT (5 g/d) Prepackaged lunch, dinners provided plus 250 g fruit and vegetables/d	14 g margarine/d containing LCT (70% rapeseed, 30% soybean oil) (5 g/d)	BW, body composition, TG, and total cholesterol
St Onge and colleagues 2003[10] Bourque and colleagues 2003[38]	C-O 2×27 d, 4-8 wk washout	17 obese women, mean age 44 y, mean BMI=32, in Montreal	30% from fat mixture containing 20% energy from MCT (~54 g/d) plus 22 mg/kg/BW stanol/sterol mixture 3 isoenergetic meals 40% energy from fat, 45% energy from CHO, and 15% energy from protein	30% energy from beef tallow	BW, body composition, TG, total cholesterol, HDL cholesterol, and LDL cholesterol
St Onge and colleagues 2003[9] St Onge and colleagues 2003[39] St-Onge and Jones 2003[40]	C-O 2×4 wk, 4-wk washout	25 overweight men, mean age 43 y, in Montreal	30% energy from structured oil containing 20% energy from MCT plus 3% stanol/sterol mixture 3 isoenergetic meals 40% energy from fat, 45% energy from CHO, and 15% energy from protein	30% energy from olive oil	BW, body composition, and blood lipid levels

(*continued on next page*)

Table. Characteristics of randomized controlled trials examining the effect of medium-chain triglycerides on weight, body composition, and blood lipid levels selected for this meta-analysis (*continued*)

Study[a]	Methods and duration	Participants	Diet		Outcomes
			Intervention	Control	
Roynette and colleagues 2008[35,o] Rudkowska and colleagues 2006[41,o]	SB C-O 2×6 wk, 4-8 wk washout	32 hypercholesterolemic, overweight men, 18-45 y, in Montreal	30% energy from structured oil containing 13% energy from MCT (48 g/d) plus 6%-10% sterol esters	30% energy from olive oil	BW and body composition
			3 isoenergetic meals 40% energy from fat, 45% energy from CHO, and 15% energy from protein		
St Onge and Bosarge 2008[32] St Onge and colleagues 2008[42]	DB P 16 wk	49 overweight adults, 19-50 y, in Birmingham, AL	12% energy from MCT, women: 18 g/d; men: 24 g/d	12% energy from olive oil	BW, body composition, TG, total cholesterol, HDL cholesterol, LDL cholesterol
			Study muffins (10 g) and cooking oil (8-14 g)		
Xue 2009[29,o] Xue 2009[43,o] Liu 2009[37,o] Zhang 2010[44,o]	DB P 8 wk	101 adults, BMI >22, high triglycerides, China	25-30 g/d MLCT oil containing <2% energy from MCT, 3.25-3.9 g/d	25-30 g/d LCT oil	BW, body composition, TG, total cholesterol, HDL cholesterol, and LDL cholesterol

[a]Where >1 article is published for the study, the primary study is listed first.
[b]DB: double-blind.
[c]P=parallel.
[d]CHO=carbohydrate.
[e]LCT=long-chain triglycerides.
[f]MCT=medium-chain triglycerides.
[g]BW=body weight.
[h]SB=single blind.
[i]BMI=body mass index.
[j]TG=triglycerides.
[k]HDL=high-density lipoprotein.
[l]LDL=low-density lipoprotein.
[m]C-O=crossover.
[n]Norovital.
[o]Risk of commercial bias, where authors are also employees of the company supplying the MCT and ownership of data and publishing is not declared.
[p]SMLCT=structured medium long-chain triglycerides.
[q]MLCT=medium long-chain triglycerides.

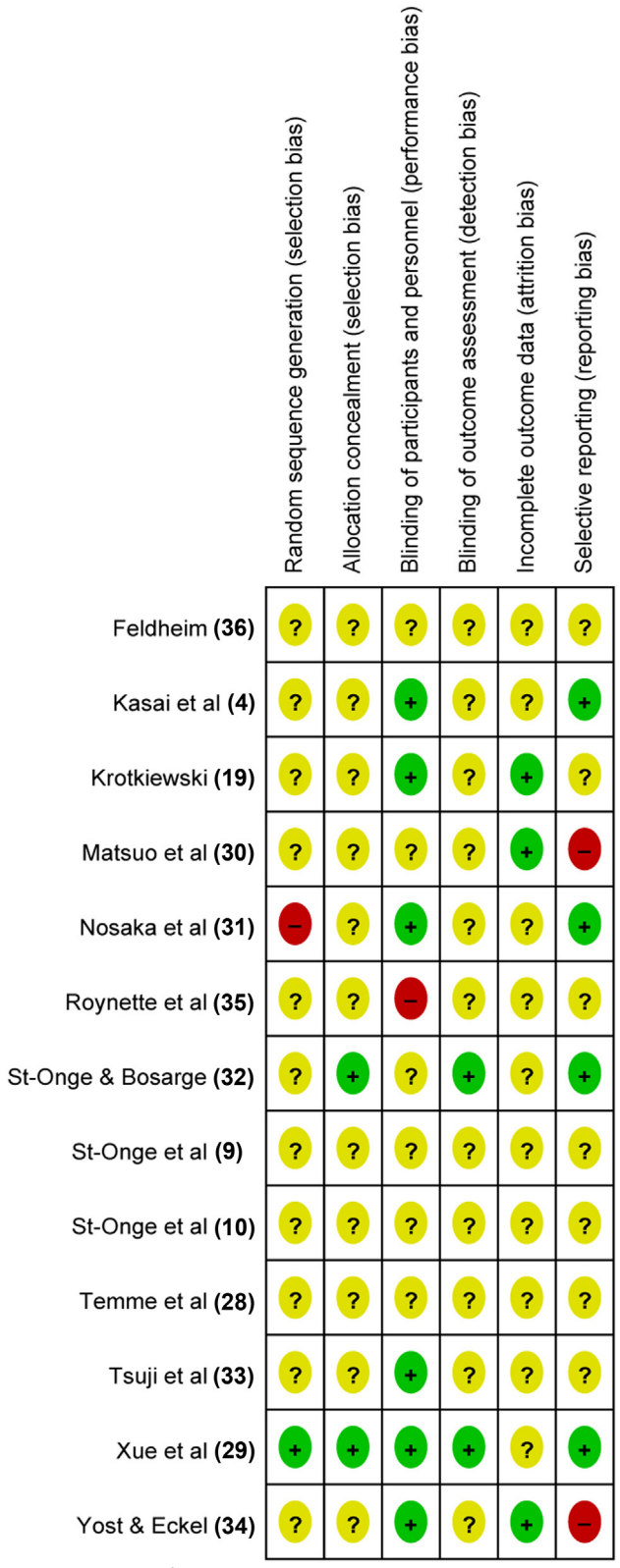

Figure 2. Risk of bias table showing judgments on each risk factor for each primary study included in meta-analysis. +=low risk (green). ?=unclear risk (yellow). −=high risk (red).

RESULTS

The literature search identified 701 possible studies, 675 were eliminated based on title and abstract because they did not meet the inclusion criteria (Figure 1). A full-text assessment was completed on 26 articles: 21 articles represented 13 RCTs that met the inclusion criteria and are described in the Table. The five excluded studies are referenced in Figure 1.

The final analysis included 239 and 250 individuals in the intervention or control diet, respectively, for nine parallel studies[4,19,28-34] and 90 participants in four crossover trials.[9,10,35,36] Eight studies provided information by sex: four crossover studies[9,10,35,36] with 42 women and 48 men, and four parallel studies[19,29,30,37] of 94 women and 80 men. Study length ranged from 27 days to 16 weeks, equating to 10 diet weeks/person. Body mass index ranged from 20 to >30. Intake of MCT ranged from <1% to 24% of energy intake. Body fat was assessed in nine studies using a variety of methods: air-displacement method,[4,31,33] bioelectrical impedance analyzer,[29] computed tomography,[4,29,31-33] dual-energy x-ray absorptiometry,[19,32] and magnetic resonance imaging.[9,10,35] Eleven RCTs measured blood lipid levels.[4,9,10,19,28-33,35] Differing controls were used but the primary controls were monounsaturated fatty acid and polyunsaturated fatty acid sources; for example, soybean oil, rapeseed oil, and olive oil containing 18 carbon atoms.[4,9,28-35] Two studies used saturated fatty acid sources, including myristic acid (C14:0)[28] and beef tallow (C15:0, C16:0),[10] and a further two studies described the control as an LCT[19,36] (see the Table).

Quality Assessment of Studies

No studies were excluded based on quality of study, although many studies did not report sufficient information for a clear bias assessment. The risk of bias for each study is summarized in Figure 2. A major possible source of bias was selection bias. Only one study detailed a method through random numbers[29]; other studies mentioned randomization, but no method was given. Allocation concealment was seen in two using central allocation[29] and opaque containers.[32] Performance bias had the best result: six studies had double-blinding,[4,19,29,31,33,34] one study was single-blind,[35] and remaining studies did not discuss blinding[9,10,30,36] or there was a possibility the blinding could have been broken[32] or unclear.[28] Detection bias was eliminated in two studies[29,32] and not mentioned in 11 studies.[4,9,10,19,28,30,31,33-36] Attrition bias was low in three studies with no dropouts.[19,30,34] The remaining studies did a completers analysis and it is unlikely the results would substantially depart from original allocation.[4,9,10,28,29,31-33,35,36] Selective reporting bias was seen in two studies: one study failed to report subgroups of in- and outpatients with differing study durations[34]; another study did not report hip and waist circumference measurements taken and provided insufficient information for body weight to be included in the meta-analysis.[30] Low risk of reporting bias was seen in four studies where all expected outcomes were reported.[4,29,31,32] The remaining studies had unclear risk because some expected outcomes might not have been reported.[9,10,19,28,33,35,36] Commercial bias was found in six studies.[4,29-31,33,35]

The risks of bias from four crossover trials were assessed.[9,10,35,36] Study design was considered suitable for weight maintenance and the washout period was sufficient at

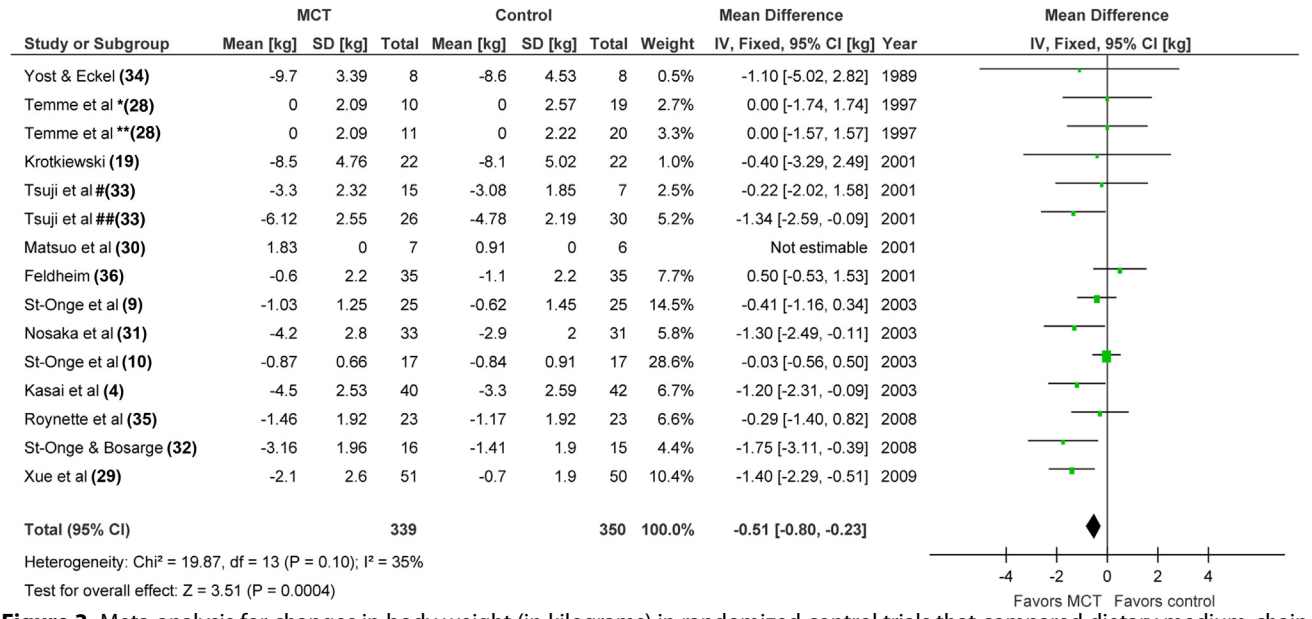

Figure 3. Meta-analysis for changes in body weight (in kilograms) in randomized control trials that compared dietary medium-chain triglycerides (MCTs) with a longer-chain triglyceride (control) shows a favorable effect of MCT intervention on body weight. *Oleic acid as control. **Myristic acid as control. #Body mass index <23. ##Body mass index ≥23. IV=inverse variance. SD=standard deviation. NOTE: Information from this figure is available online at www.andjrnl.org as part of a PowerPoint presentation.

4 or 8 weeks, although one study only had a 2-week washout.[36] The risk of a carryover effect was not clear, nor were data from the first period available. In two cases, the SD of the mean difference had to be imputed.[10,36]

All studies, except one,[30] had methods to check dietary compliance. Ten studies provided some or all food to participants.[4,9,10,19,31-36] One study monitored physical activity levels,[31] whereas eight studies advised participants to maintain current activity levels.[4,9,10,28,29,33-35] Four studies limited alcohol consumption.[4,31,33,34] Three studies were hypocaloric.[19,32,34] Three studies added phytosterols to the intervention oil[9,10,35] and one added psyllium.[34]

Effect of MCTs on Body Composition in Adults

Body Weight. Twelve studies—eight parallel[4,19,28,29,31-34] and four crossover trials[9,10,35,36]—recorded body weight outcomes (Figure 3). The weighted mean difference for body weight significantly favored MCT with small effect (−0.51 kg [95% CI −0.80 to −0.23 kg]). Moderate heterogeneity was seen in the meta-analysis for body weight (I^2=35%; P=0.10). There did not appear to be one study driving the moderate heterogeneity. Sensitivity analyses did not change this result except for study design where parallel vs crossover trials removed the significance for all the subgroup analyses, perhaps due to loss of statistical power. Furthermore, a post hoc sensitivity analysis of hypocaloric studies[19,32,34] did not alter the results. Subgroup analysis identified significant differences between duration, dose, and commercial biased groups. Studies ≥12 weeks showed greater weight loss than those <12 weeks (P=0.004). More weight was lost with doses <8% energy compared with doses >16% energy (P=0.03). More weight was lost in the commercially biased trials compared with the noncommercially biased trials (P=0.001).

Waist and Hip Circumference. Waist[4,29,31-33] and hip circumferences[4,29,31] were recorded in five and three parallel studies as shown in Figure 4. The weighted mean difference significantly favored MCT in waist circumference with medium effect (−1.46 cm [95% CI −2.04 to −0.87 cm]) and hip circumference with small effect (−0.79 cm [95% CI −1.27 to −0.30 cm]). Heterogeneity was not present for either waist (I^2=0%; P=0.71) or hip circumference (I^2=0%; P=0.87) (Figure 4). A subgroup analysis did not show any intergroup differences and results were not affected by a sensitivity analysis.

Total body, Subcutaneous, and Abdominal Visceral Fat. Nine studies—six parallel[4,19,29,31-33] and three crossover trials[9,10,35]—recorded outcomes for total body fat. Seven studies—four parallel[4,29,31,33] and three crossover trials[9,10,35]—measured outcomes for total subcutaneous fat. Six studies—five parallel studies[4,29,31-33] and one crossover trial[35]—recorded outcomes for visceral fat. Studies are detailed in Figure 5. The SMD significantly favored MCT in total body fat with small effect (SMD=−0.39 [95% CI −0.57 to −0.22]), total subcutaneous fat with medium effect (SMD=−0.46 [95% CI −0.64 to −0.27]) and visceral fat with medium effect (SMD=−0.55 [95% CI −0.75 to −0.34]). Heterogeneity was not present in total body fat (I^2=0%; P=0.62), total subcutaneous fat (I^2=20%; P=0.27), or visceral fat (I^2=0%; P=0.53). A subgroup analysis did not show any intergroup differences and results were not affected by a sensitivity analysis.

Effect of MCTs on Blood Lipid Levels in Adults

Three crossover studies[9,10,35] included phytosterols in the intervention oil; they have been excluded from analysis of blood lipids because phytosterols may confound results.[42]

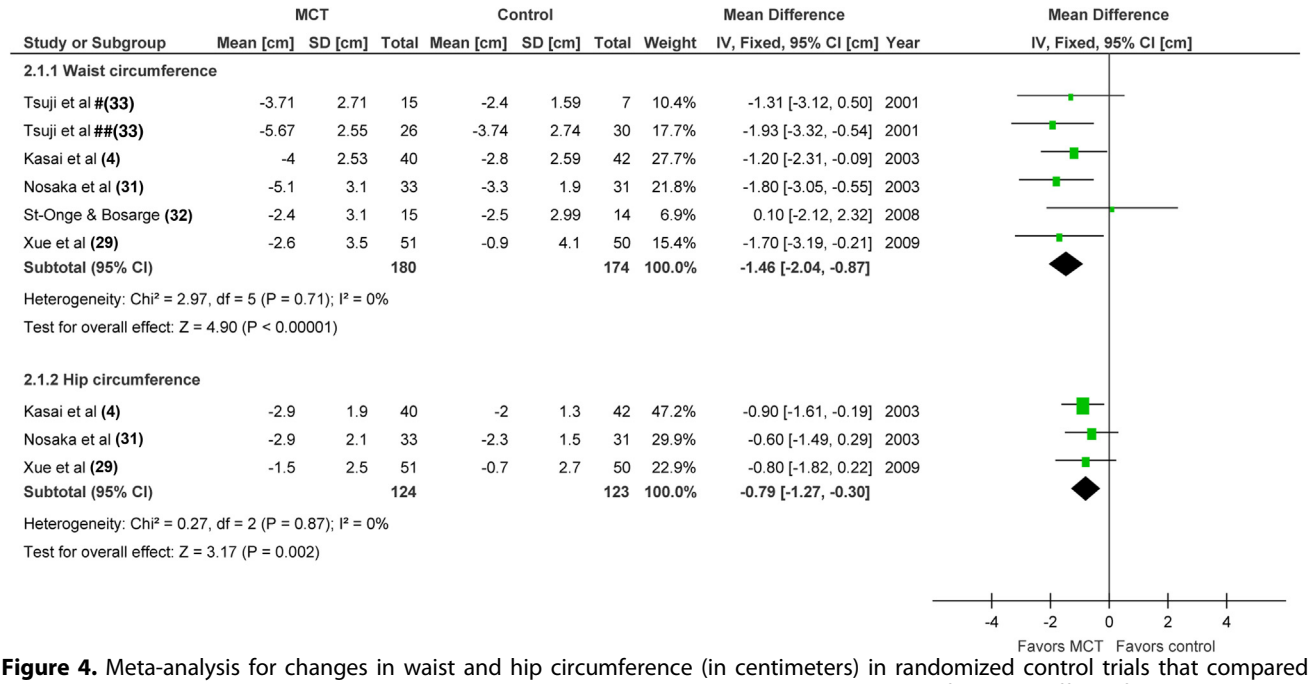

Figure 4. Meta-analysis for changes in waist and hip circumference (in centimeters) in randomized control trials that compared dietary medium-chain triglycerides (MCTs) with a longer-chain triglycerides (control) shows a favorable effect of MCT intervention on waist and hip circumference. #Body mass index <23. ##Body mass index ≥23. IV=inverse variance. SD=standard deviation.

Similarly, baseline lipid levels may affect lipid responses to dietary interventions[50]; because the study by Xue and colleagues[29] was the only study conducted on patients with hypertriglyceridemia this study was excluded from the blood lipids analysis.

Seven parallel studies[4,19,28,30-33] recorded outcomes for TG with no heterogeneity (I^2=0%; P=0.77). MCTs did not affect TG (0.00 mg/dL; 95% CI −8.04 to 8.04 mg/dL [0.00 mmol/L; 95% CI −0.09 to 0.09 mmol/L]). Seven parallel[4,19,28,30-33] studies recorded outcomes for total cholesterol. MCT did not affect total cholesterol (−2.33 mg/dL; 95% CI −6.84 to 2.18 mg/dL [−0.06 mmol/L; 95% CI −0.18 to 0.06 mmol/L]) and no heterogeneity (I^2=0%; P=0.46) was present. Four parallel[4,28,31,32] studies recorded outcomes for LDL cholesterol with small heterogeneity (I^2=36%; P=0.18). MCT did not affect LDL cholesterol levels (−2.91 mg/dL; 95% CI −7.62 to 1.79 mg/dL [−0.08 mmol/L; 95% CI −0.20 to 0.05 mmol/L]). Five parallel studies[4,28,30-32] recorded outcomes for HDL cholesterol with no heterogeneity (I^2=0%; P=0.54). MCT did not affect HDL cholesterol levels (−1.35 mg/dL; 95% CI −3.02 to 0.31 mg/dL [−0.03 mmol/L; 95% CI −0.08 to 0.01 mmol/L]). Studies and results are summarized in Figure 6. Subgroup analyses did not show any intergroup differences and sensitivity analyses did not alter any results. Furthermore, a post hoc sensitivity analysis of studies using a saturated fatty acid as a control[10,28] did not alter the results.

Publication Bias

In this meta-analysis, publication bias was not detected for weight loss outcomes, but there was a trend for publication bias in total body fat outcomes (P=0.05) (Figure 7).

DISCUSSION

The findings from our meta-analysis suggest consuming MCTs as part of a diet compared with LCTs may result in a small average reduction in body weight of 0.51 kg (range=0.80 to 0.23 kg) over an average 10-week period. Waist and hip circumferences, total body fat, subcutaneous fat, and visceral fat were also significantly reduced and no changes were seen in TG, total cholesterol, LDL cholesterol, and HDL cholesterol when dietary MCT was compared with LCT. Although the reduction in body weight was small, it may be clinically relevant. When body weight is decreased by 1 kg, the associated risk of developing diabetes is reduced by 16%.[51] Nonetheless, several factors may have affected the quality of the meta-analysis results, which limits firm conclusions. The study designs in this meta-analysis were highly variable, including trials with very-low-calorie diets in obese women,[19,34] with normal-weight individuals,[29,33] or obese and overweight individuals where energy intake was highly controlled for weight maintenance[4,9,10,33,35] or on free-feeding weight-loss diets.[32] Dosages of MCT varied considerably from <1% percent energy (2 g/day) up to >20% energy (54 g/day) and duration of studies varied from 4 to 16 weeks. The reporting of studies was incomplete with few studies providing sufficient information to make a clear bias assessment. In addition, 6 out of 13 studies were identified with high risk of commercial bias.[4,29-31,33,35]

A recent systematic review of acute, short- and long-term studies published between 2000 and 2010 concluded that dietary intake of MCTs may be associated with improved body composition and increased energy expenditure without obvious effects on food intake.[21] The authors indicated that the results were inconclusive and further studies are

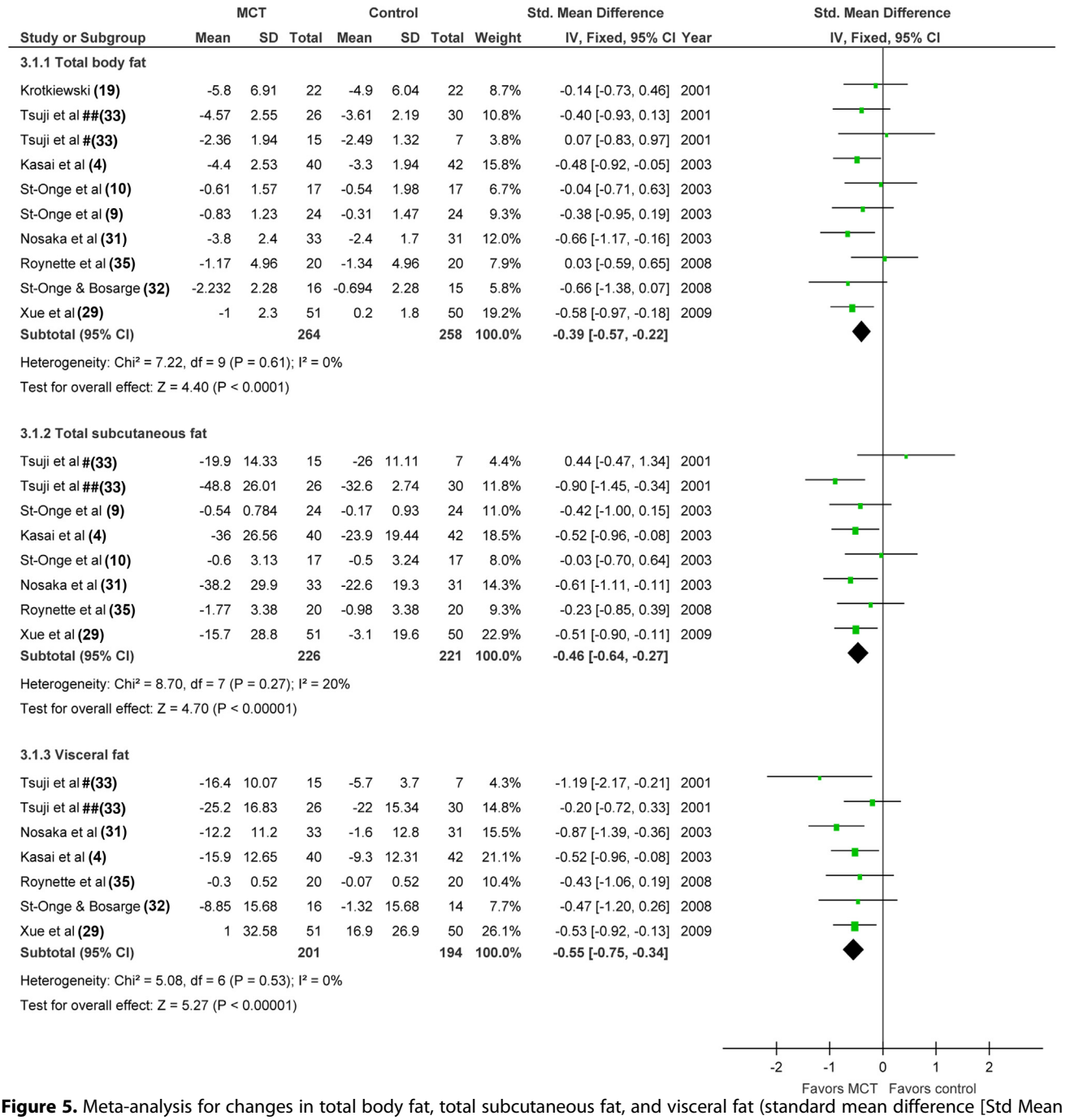

Figure 5. Meta-analysis for changes in total body fat, total subcutaneous fat, and visceral fat (standard mean difference [Std Mean Difference]) in randomized control trials that compared dietary medium-chain triglycerides (MCTs) with a longer-chain triglycerides (control) shows a favorable effect of MCT intervention on body fat. #Body mass index <23. ##Body mass index ≥23. IV=inverse variance. SD=standard deviation.

warranted to determine adequate amounts and long-term side effects of MCTs. Our systematic review focused on chronic (>3 weeks) RCTs published until March 2014 comparing MCT with LCT on weight loss, body composition, and lipid profiles as secondary outcomes and included a meta-analysis of the outcomes. Although no new studies have been published since 2010, our review identified six RCTs[28-31,34,36] that were not included in the previous systematic review. Our systematic review furthermore provides a comprehensive critical appraisal of the current evidence and aims to identify gaps and areas of further research needed before recommendations for intake of MCTs to facilitate body weight management can be made.

All research is susceptible to bias, but industry-funded trials tend to report results favoring their own products[52] and possibly lead to publication bias. Industry funding is

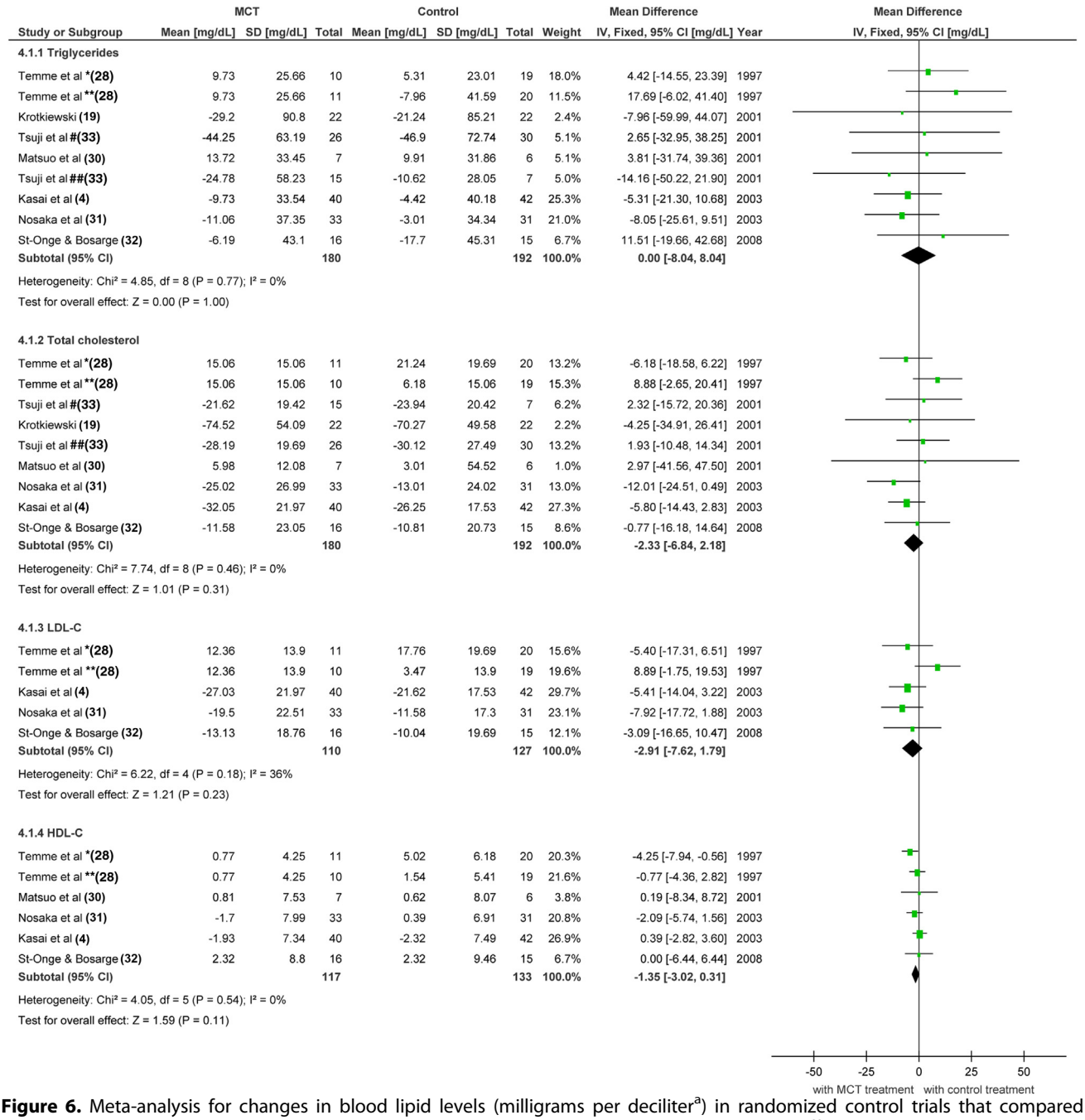

Figure 6. Meta-analysis for changes in blood lipid levels (milligrams per deciliter[a]) in randomized control trials that compared dietary medium-chain triglycerides (MCT) with a longer chain triglyceride (control) shows no differences with MCT intervention. Four studies were excluded from the analysis: three contained phytosterols in the intervention oil and one had participants with hyperlipidemia. [a]To convert mg/dL triglycerides to mmol/L, multiply mg/dL by 0.0113. To convert mmol/L triglyceride to mg/dL, multiply mmol/L 88.6. Triglyceride of 159 mg/dL=1.80 mmol/L. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.0259. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. Cholesterol of 193 mg/dL=5.00 mmol/L. *Oleic acid as control. **Myristic acid as control. #Body mass index <23. ##Body mass index ≥23. IV=inverse variance. SD=standard deviation.

critical for the advancement of nutrition science and not all industry-funded trials are biased, particularly if parties follow the guiding principles for industry-funded research,[53] including (but not exhaustively) the conduct of transparent and objectively designed research according to accepted principles of scientific inquiry, the control of both study design and research remaining with the scientific investigators, freedom and obligation to publish the findings, and full disclosure of all financial interest. The six trials identified were cowritten by employees of the suppliers of the MCT trial products. As coauthors, it is assumed they contributed substantially to the design of the research; the

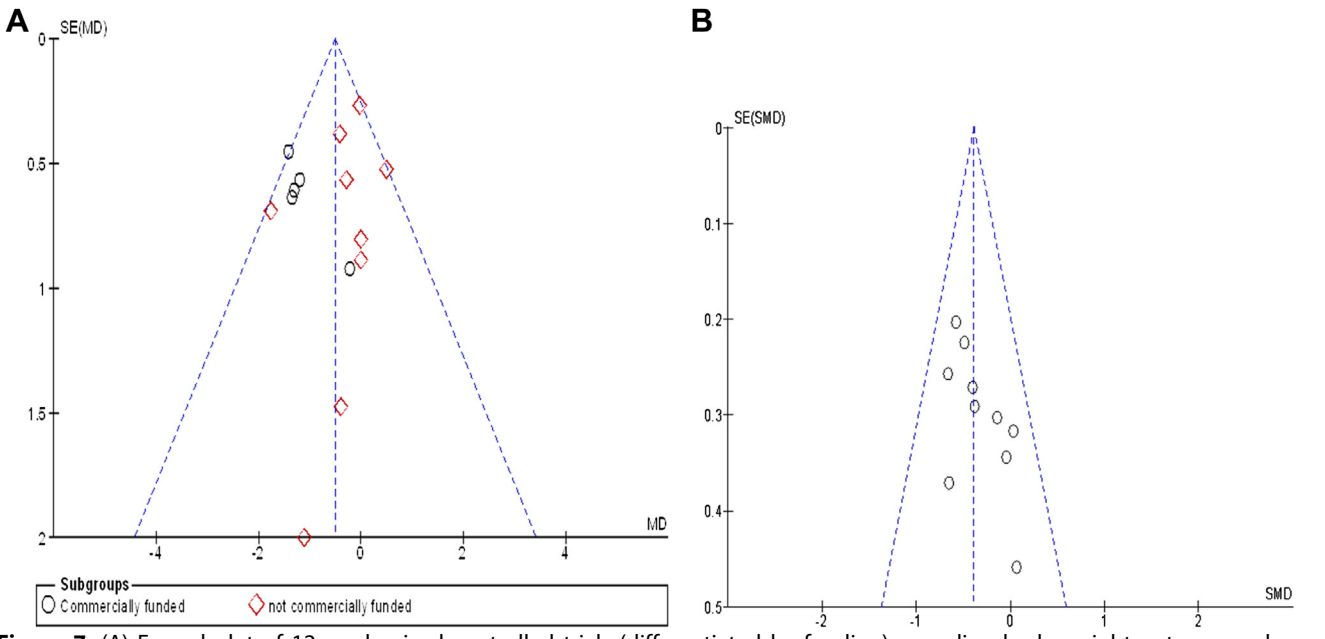

Figure 7. (A) Funnel plot of 12 randomized controlled trials (differentiated by funding) recording body weight outcomes when dietary medium-chain triglycerides were compared with a longer-chain triglyceride (control). An Egger linear regression analysis showed a symmetrical funnel plot (P=0.35). (B) Funnel plot of 10 randomized controlled trials recording total body fat outcomes when dietary medium-chain triglycerides were compared with a longer-chain triglyceride (control). An Egger linear regression analysis showed a trend toward an asymmetrical funnel plot (P=0.05).

acquisition, analysis, and interpretation of data; decision to publish and the drafting; revision; and approval of the final manuscript for submission, which may conflict with the guiding principles described above. Although these studies were generally well designed (randomized, controlled, blind, and sufficient duration), the reporting was poor resulting in unclear bias assessments for all, except Xue and colleagues.[29] It is interesting to note these studies were most likely to show a significant improvement in body weight despite being conducted on normal/overweight samples (four of five studies). They also provided the smallest doses, ≤4% energy from MCT, and were the longest in duration (four studies being 12 weeks' duration),[4,30,31,33] both factors shown in subgroup analysis to most favorably affect body weight.

The studies using the smallest doses[4,29,31,33] of ≤4% of energy (1.7 to 10 g/day), showed significant improvements in body weight (~−1.2 kg), body fat (~−1.0 kg), subcutaneous fat (~−10.3 cm^2), and visceral fat (~−8.9 cm^2) over 8 to 12 weeks compared with LCT and were conducted in Japanese[4,31,33] and Chinese[29] participants of normal weight. However, Tsuji and colleagues[33] only showed significant improvements in participants with body mass index ≥23. According to Kasai and colleagues,[4] 2 g/day MCT was small, yet 8 to 50 times the usual intake by Japanese people (0.2 g/day), and perhaps sufficient to accelerate lipid metabolism in human beings. Feldheim,[36] with young European women of normal body mass index, also used a small dosage of approximately 12.5 g/day (~5% energy) MCT in an ad libitum diet, but did not see significant changes in body weight. However, the energy and fat consumption were greater (P<0.01 and P<0.001) and protein consumption lower (P<0.05) in the MCT group compared with the LCT group, which could have confounded the results.

In contrast, St Onge and colleagues showed that large doses of 20% energy from MCT (~54 g/day), as part of a strictly controlled weight maintenance diet in Canadian overweight men[9] and obese women,[10] resulted in increased energy expenditure and fat oxidation in both men and women, although weight loss was not significant compared with LCT. Similarly, Roynette and colleagues,[35] using the same study design as St Onge and colleagues[9,10] but a reduced dose (13% energy, 48 g/day), did not show significant differences in body weight and body composition between MCT and LCT in overweight men. The differences in weight loss between MCT and LCT in these trials may have been greater if the energy intake of the diets were not strictly controlled to maintain weight; instead, free feeding may allow the satiety effects of MCT to operate. The total fat intake in these studies were high at 40% energy compared with recommended intakes of 30% energy (US Department of Agriculture National Nutrient Database), which may have affected the outcomes. In addition, olive oil was used as control in two of these studies.[9,35] Olive oil consumption has been shown to increase fat oxidation[54] and lead to beneficial body composition changes[55,56] and could, therefore, have diminished the differences between MCT and control. St Onge and Bosarge[32] conducted a follow-up weight-loss trial in overweight men and women with a moderate amount of MCT (~12% energy: 18 g/day for women, 24 g/day for men). This dose was used by Dulloo and colleagues,[11] who showed 15 to 30 g/day significantly enhanced energy expenditure. St Onge and Bosarge[32] presented a decrease in body weight (−1.75 kg [95% CI −3.11 to −0.39 kg]) over 16 weeks compared with olive oil. In this trial, participants were allowed free feeding to capture the satiety effect of MCTs. However, the authors did not report dietary intake data to determine whether the energy intake was reduced in the MCT group. Temme and colleagues[28]

conducted an ad libitum dietary study in the Netherlands on healthy adults (body mass index ranged from 20 to 30) with 10% energy from MCT (23.7 g/day) compared with either myristic acid margarine or sunflower oil margarine and failed to show weight loss effects in either trial arm, but the study's primary aim was to investigate the effects of MCT on lipid profiles.

Two studies[19,34] involving very-low-calorie diets (800 kcal/day and 579 kcal/day) containing MCT doses of 24% energy (21 g/day) and 13% energy (9 g/day) in obese women were unable to show any effects on body weight and the results were highly variable with wide 95% CIs (Figure 3). Krotkiewski[19] showed greater reductions in body weight and body fat, a sparing effect on lean mass, and decreased hunger feelings that paralleled higher increases in ketone bodies with MCT compared with LCT after 2 weeks, but not after 4 weeks. They argued the effects of MCT might be transient, which is disputed by the results of our meta-analysis. The role of MCTs in very-low-calorie liquid diets; therefore, needs further investigation.

It is important to consider effects on lean mass and whether MCT could prevent or reduce the reduction in lean mass typically associated with weight-reducing diets. However, few studies have measured the effects of MCT on lean mass with inconsistent effects. St Onge and Bosarge[32] in their weight loss study showed lean mass was higher in the olive oil control group than in the MCT group at the end of the study (−0.93±0.41 kg), whereas Krotkiewski[19] showed reduced loss of lean mass with MCT compared with LCT with their very-low-calorie diet.

With regard to the investigation of MCT on lipid profiles, it must be noted that blood lipid levels were a secondary outcome and not included as a search term; therefore, some key studies may have been omitted and firm conclusions cannot be drawn from this meta-analysis. This analysis has at least illustrated high doses of MCT do not adversely affect lipid profiles. This should be further investigated in a meta-analysis specifically designed to answer this research question.

One consistent quality from the studies is the method of dietary intervention. Many studies provided food to control energy and dietary fat intake, minimizing confounders and the risk of blinding being broken. Another strength of the trials included assessment of body composition to support body weight loss, although few studies measured lean mass. However, a limitation of the studies included the large variation of body mass index within studies and a failure to control for baseline body weight in many studies. In addition, the study of dietary MCT and weight loss has centered principally around two research groups: one in Japan[4,30,31,33] and the other from Canada[9,10,35]; thus, outcomes may not be applied to the general population. Subgroup analyses are observational only as they are not based on randomized comparisons,[26] so subgroup results should be used with caution. A limitation of this meta-analysis was that the retrieval, extraction, and bias analyses of the studies was conducted by one author and verified by the second author. However, the verification involved rigorous scrutiny of the information and it is unlikely to have affected the quality of the meta-analysis.

The replacement of LCT with MCT in the diet could potentially result in small decreases in body weight and composition without adversely affecting lipid profiles. However, several factors affected the quality of the meta-analysis results: inadequate reporting made it difficult to assess the quality of the trials; several studies were at high risk of commercial bias; and the study designs varied considerably with regard to dosage, duration, and control of energy intake. Further research is required by other independent research groups using large, robustly designed studies of sufficient duration (at least 12 weeks), with different MCT doses in different food matrixes, different populations, different body weight status groups (normal weight vs overweight/obese), sex, and age groups also capturing pre- and postmenopausal women. The effect of MCT on the fat:lean mass also needs to be investigated, particularly in weight loss studies and older adults. The long-term safety of MCT-enriched foods also need to be ascertained. Finally, all studies should be reported according to the Consolidated Standards of Reporting Trials guidelines (http://www.consort-statement.org/), which provides a set of evidence-based recommendations for reporting randomized trials in a standard way that facilitates transparent and complete reporting that aids in their critical appraisal and interpretation.[57]

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

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EXHIBIT C

Dietary Fats and Cardiovascular Disease

A Presidential Advisory From the American Heart Association

ABSTRACT: Cardiovascular disease (CVD) is the leading global cause of death, accounting for 17.3 million deaths per year. Preventive treatment that reduces CVD by even a small percentage can substantially reduce, nationally and globally, the number of people who develop CVD and the costs of caring for them. This American Heart Association presidential advisory on dietary fats and CVD reviews and discusses the scientific evidence, including the most recent studies, on the effects of dietary saturated fat intake and its replacement by other types of fats and carbohydrates on CVD. In summary, randomized controlled trials that lowered intake of dietary saturated fat and replaced it with polyunsaturated vegetable oil reduced CVD by ≈30%, similar to the reduction achieved by statin treatment. Prospective observational studies in many populations showed that lower intake of saturated fat coupled with higher intake of polyunsaturated and monounsaturated fat is associated with lower rates of CVD and of other major causes of death and all-cause mortality. In contrast, replacement of saturated fat with mostly refined carbohydrates and sugars is not associated with lower rates of CVD and did not reduce CVD in clinical trials. Replacement of saturated with unsaturated fats lowers low-density lipoprotein cholesterol, a cause of atherosclerosis, linking biological evidence with incidence of CVD in populations and in clinical trials. Taking into consideration the totality of the scientific evidence, satisfying rigorous criteria for causality, we conclude strongly that lowering intake of saturated fat and replacing it with unsaturated fats, especially polyunsaturated fats, will lower the incidence of CVD. This recommended shift from saturated to unsaturated fats should occur simultaneously in an overall healthful dietary pattern such as DASH (Dietary Approaches to Stop Hypertension) or the Mediterranean diet as emphasized by the 2013 American Heart Association/American College of Cardiology lifestyle guidelines and the 2015 to 2020 Dietary Guidelines for Americans.

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Key Words: AHA Scientific Statements ■ blood cholesterol ■ cardiovascular diseases, atherosclerosis ■ cholesterol, LDL ■ dietary fats ■ fatty acids, saturated ■ fatty acids, unsaturated

Cardiovascular disease (CVD) is the leading global cause of death, accounting for 17.3 million deaths per year, comprising 31.5% of total global deaths in 2013. Nearly 808 000 people in the United States died of heart disease, stroke, and other CVDs in 2014, translating to about 1 of every 3 deaths. The annual direct and indirect costs of these deaths total more than $316.1 billion, including health expenditures and lost productivity.[1] Preventive treatment that reduces CVD by even a small percentage can substantially reduce, nationally and globally, the number of people who develop CVD and the costs of caring for them.

Since 1961, the American Heart Association (AHA) has recommended reduction in dietary saturated fat to reduce the risk of CVD.[2,3] The purpose of this AHA presidential advisory on dietary fats and CVD is to review and discuss the scientific evidence, including the most recent studies, on the effects on CVD of dietary saturated fat and its replacement by other types of fats and carbohydrates. A presidential advisory is initiated by the AHA president to address a topic of special current importance. This report discusses the major classes of dietary fatty acids, except for the very-long-chain n-3 fatty acids in fish, which are covered by other AHA reports.

The scientific rationale for decreasing saturated fat in the diet has been and remains based on well-established effects of saturated fat to raise low-density lipoprotein (LDL) cholesterol, a leading cause of atherosclerosis[4]; to cause atherosclerosis in several animal species, especially nonhuman primates[5]; to clear the atherosclerosis in animals[6] when it is reduced in the diet; and likewise to reverse atherosclerosis in humans.[7,8] In addition, reducing saturated fat and replacing it with polyunsaturated fat in randomized controlled trials has reduced the incidence of CVD.[9,10] Populations with very low saturated fat intake such as in East Asian and Mediterranean countries have very low rates of CVD,[11] and members of many single populations who have low saturated and high unsaturated fat intake have lower future incidence of CVD compared with those with high saturated and low unsaturated fat intake.[12] The current AHA/American College of Cardiology guideline is to decrease intake of saturated fat to 5% to 6% of total daily energy (calorie) intake for individuals with elevated LDL cholesterol concentration.[3] The 2015 to 2020 Dietary Guidelines for Americans recommend consuming <10% of calories from saturated fat for the general population and replacing saturated fat with unsaturated fat.[13] The average intake of saturated fat in adults in the United States is 11% of total daily energy intake[13,14]; only about 5% of adults consume <7%, and 30% to 40% consume <10%.[14] Thus, most adults need to reduce saturated fat to reduce their risk of CVD. The implementation strategy recommended to achieve this reduction is to shift food choices from those high in saturated to those high in polyunsaturated and monounsaturated fats.[3,13]

In the past few years, meta-analyses of observational studies and randomized clinical trials have come to discordant conclusions about the relationship between dietary saturated fat and risk of CVD.[9,10,12,15–17] This has created confusion among patients, their physicians, and the public. In this article, we analyze and discuss the methodology and interpretation of results reported by these researchers and the reasons for the divergent findings.

SUMMARY OF CONCLUSIONS

Dietary saturated fat, like any macronutrient, supplies energy (calories) to the diet. In randomized clinical trials on saturated fat, the group that is assigned a diet lower in saturated fat is taught how to replace it with foods higher in ≥1 other macronutrients, typically carbohydrates or unsaturated fats, to maintain the same total energy intake. Other trials, often called controlled feeding trials, actually provide to the research participants their assigned diet high or low in saturated fat balanced with a similar amount of energy from another macronutrient. Essential to the interpretation of the results from these trials (and the reason for the divergent results in meta-analyses noted above) is the macronutrient composition of the comparator diet. Clinical trials that used polyunsaturated fat to replace saturated fat reduced the incidence of CVD.[9,10] In contrast, trials that used mainly carbohydrates to replace saturated fat did not reduce CVD. However, the types of carbohydrate-containing foods were often unspecified and typically included sugar and other refined carbohydrates to maintain energy balance. Evidence from prospective observational studies indicates that carbohydrates from whole grains reduce CVD when they replace saturated fat.[18]

Prospective observational studies, also called cohort studies, are conducted in large populations in which dietary intake is assessed at the beginning of the study and in some studies reassessed repeatedly during the follow-up periods, and CVD is assessed at various points during follow-up. In prospective observational studies, the participants eat whatever diet they themselves choose, and the researchers request that participants report their recent or past dietary history. Research participants in observational studies who eat a large amount of saturated fat eat less of various other macronutrients, usually carbohydrates, unsaturated fat, or both, to maintain energy intake. Participants who eat a comparatively small amount of saturated fat eat more carbohydrates or unsaturated fats. Because carbohydrates and unsaturated fats differ in their metabolic effects, it is necessary to evaluate the effects of low and high saturated fat intakes in the context of the replacement

macronutrient. This is easier in a clinical trial because the trial controls the dietary intake but more complicated in observational studies in which the participants control their own diets.

Meta-analyses of prospective observational studies aiming to determine the effects on CVD of saturated fat that did not take into consideration the replacement macronutrient have mistakenly concluded that there was no significant effect of saturated fat intake on CVD risk.[15,16] In contrast, meta-analyses that specifically evaluated the effect of replacing saturated fat with polyunsaturated fat found significant benefit, whereas replacing saturated fat with carbohydrates, especially refined carbohydrates, yielded no significant benefit to CVD risk.[12,17,18] Thus, again, differences in the effects of the replacement or comparator nutrients, specifically carbohydrates and unsaturated fats, are at the root of the apparent discrepancies among studies and meta-analyses on whether lowering saturated fat reduces the risk of developing CVD. In fact, the evidence to recommend reduction of saturated fat and its replacement by polyunsaturated and monounsaturated fat has strengthened as better methodology is more widely adopted for the analysis of dietary intake in observational studies.

We judge the evidence to favor recommending n-6 polyunsaturated fat, that is, linoleic acid, stronger than monounsaturated fat to replace saturated fat because of the positive results of randomized clinical trials that used polyunsaturated fat compared with the paucity of trials that used monounsaturated fat[10]; the greater relative risk reduction for polyunsaturated fats in observational studies[12,17,18]; the greater reduction in LDL cholesterol with polyunsaturated fat[4]; and the regression of atherosclerosis in nonhuman primates by polyunsaturated but not monounsaturated fat.[5] However, progress in reducing CVD would be enhanced by replacing saturated fat by either type of unsaturated fat.

FATTY ACID COMPOSITION OF FATS AND OILS

The fatty acid composition of major fats and oils in the diet is shown in the Table.[19] The main sources of saturated fat to be decreased are dairy fat (butter), lard (pork), beef tallow, palm oil, palm kernel oil, and coconut oil. Polyunsaturated fats are contained in canola oil, corn oil, soybean oil, peanut oil, safflower oil, sunflower oil, and walnuts. However, original high-linoleic varieties of safflower and sunflower oils are uncommon. High-oleic varieties of safflower and sunflower oil, olive oil, avocados, and tree nuts such as almonds, cashews, hazelnuts, pistachios, and pecans have mainly monounsaturated fats and are low in saturated fat.

CVD OUTCOMES: RANDOMIZED CLINICAL TRIALS THAT LOWERED DIETARY SATURATED FAT

The randomized clinical trial, when designed appropriately to answer the research question and executed with high quality, is the cornerstone for health and medical guidelines and policy. However, a randomized trial of a food or nutrient must achieve a biologically meaningful difference in intake between treatment and control groups and sustain it for a long enough time to deliver a valid result. Participants may find it difficult to maintain intake of a diet to which they are not accustomed and may revert to their original more familiar diet. In some trials, the difference in dietary saturated fat was maintained for many years,[20–22] but in others, the difference fell well short of planned.[23–25] In addition, the comparator nutrient that replaced saturated fat, polyunsaturated fat or carbohydrates, differed among trials. Reviewers who evaluate these trials must take into account the specific nutritional experiment that was conducted and the level of its adherence throughout the follow-up period.

Low Saturated, High Polyunsaturated Fat Diets

In the mid-1950s, 4 research groups reported that replacing saturated fat from animal products with polyunsaturated fat from vegetable oils substantially reduced serum cholesterol levels.[26–29] Soon, controlled trials followed to test whether the reduction in serum cholesterol caused by substituting polyunsaturated for saturated fat prevented CVD. We examined several recent systematic reviews and meta-analyses[9,10,16] from which we identified and here discuss 4 trials[20–22,30] that make up the core evidence on this important question on the basis of quality of study design, execution, and adherence. These trials compared high saturated with high polyunsaturated fat intake; did not include *trans* unsaturated fat as a major component; controlled the dietary intake of the intervention and control groups; had at least 2 years of sustained intake of the assigned diets; proved adherence by objective biomarkers such as serum cholesterol or blood or tissue levels of polyunsaturated fatty acids; and collected and validated information on cardiovascular or coronary disease events. The reason for the 2-year minimum duration is that changes in polyunsaturated fatty acids very slowly equilibrate with tissue fatty acid levels; it takes ≈2 years to achieve 60% to 70% of the full effect.[20,30] Trials of serum cholesterol–lowering agents show that a reduction in coronary heart disease (CHD) incidence occurs with a lag of 1 to 2 years.[31] These systematic reviews[9,10,16] together found and analyzed 6 additional trials[7,23,32–35] that replaced saturated with polyunsaturated

Table. Fatty Acid Composition of Fats and Oils

	Saturated, g/100 g			Monounsaturated, g/100 g		Polyunsaturated, g/100 g		
	Total	Lauric (12:0), Myristic (14:0), Palmitic (16:0)	Stearic (18:0)	Total	Oleic (18:1)	Total	Linoleic (18:n-6)	α-Linolenic (18:3n-3)
Canola oil	7	4	2	63	62	28	19	9
Coconut oil	82	67	3	6	6	2	2	0
Corn oil	13	11	2	28	27	55	53	1
Dairy fat (butter)	63	39	12	26	21	4	3	0
Lard (pork)	39	25	14	45	41	11	10	1
Olive oil	14	11	2	73	71	10	10	1
Palm oil	49	45	4	37	37	9	9	0
Palm kernel oil	82	72	3	11	11	2	2	0
Peanut oil	17	10	2	46	45	32	32	0
Safflower oil (high linoleic)	6	4	2	14	14	75	75	0
Safflower oil (high oleic)*	8	5	2	75	75	13	13	1
Soybean oil	16	10	4	23	23	58	50	7
Sunflower oil (high linoleic)	10	6	4	20	20	66	66	0
Sunflower oil (high oleic)*	10	5	4	84	83	4	4	0
Tallow (beef)	50	30	19	42	36	4	3	1

A zero value equals <0.5 g/100 g.
*Primary safflower and sunflower oils of commerce.
Data from US Department of Agriculture food composition tables.[19]

fat but did not have ≥1 of these characteristics crucial to testing the hypothesis. We also discuss these "noncore" trials and evaluate their potential impact on the overall result on dietary saturated and polyunsaturated fat and risk of CVD.

Core Trials on Replacing Saturated With Polyunsaturated Fat

The Wadsworth Hospital and Veterans Administration Center in Los Angeles (Dayton et al[20]) conducted a high-quality, double-blind, well-controlled trial. There were 846 men with a mean age of 65 years, and 30% had CVD. The experimental diet used corn, soybean, safflower, and cottonseed oils, all high in polyunsaturated linoleic acid, to replace saturated fat in the control diet. The participants were served their meals at the center, with each diet group in a separate dining room. Adherence was confirmed by objective measures demonstrating enrichment with linoleic acid in blood, adipose tissue, and atherosclerosis specimens in the coronary arteries and aorta. Moreover, the investigators established double-blind conditions. The average duration was 8 years. The experimental diet reduced serum cholesterol by 13%. There were 20% fewer primary events, myocardial infarction or sudden death, in the diet group than in the control group, not a statistically significant difference. The diet significantly reduced the CVD end point, definite myocardial infarction, sudden death, or ischemic stroke, by 34% (P=0.04) and total CVD events by 31% (P=0.01). There were 41% fewer men who had an ischemic stroke in the diet group than in the control group (P=0.055).

By contemporary standards, the trial needed more participants to reach a definitive conclusion. However, the strict dietary control and 8-year-long intervention period ensured both a large difference in the dietary fatty acid intakes and enough CVD events to reach a statistically significant treatment effect for the secondary CVD outcomes, which were more highly powered because of their larger numbers of events.

The Oslo Diet-Heart Study[21] assigned at random 412 men who had had a myocardial infarction to either a control group who continued their usual high-saturated fat diet or an experimental group who changed to a low saturated, high polyunsaturated fat diet. The men in the experimental group and their wives were taught in their homes how to select and prepare foods that were low in saturated fat and high in polyunsaturated vegetable oils. The polyunsaturated fat diet lowered serum cholesterol by 14% (41 mg/dL), thereby confirming adherence, and this effect was sustained throughout the 5-year trial. The polyunsaturated fat diet significantly reduced the primary outcome, recurrent myocardial infarction and new cases of angina pectoris or sudden death, significantly by 29% (P=0.011). Among the components of the primary outcome, myocardial infarction and angina pectoris were significantly reduced by 37% and 66%, respectively, whereas incidence of sudden death was the same in both groups. The end point, myocardial

infarction or sudden death, was reduced by 25% (*P*=0.05). There were fewer cardiovascular deaths in the experimental group by 27% (*P*=0.09). The low saturated, high polyunsaturated fat group continued to experience reduced cardiovascular mortality compared with the high saturated fat control group for an additional 6 years after the trial ended.

The British Medical Research Council compared a diet containing soybean oil, 86 g/d, with a diet with saturated fat from animal products in 393 men after myocardial infarction.[22] They were instructed to drink half the soybean oil allotment with fruit juice and use half in cooking, and they were counseled on how to reduce the saturated fat in their diet so that the total fat contents of the intervention and control groups were similar. Replacing animal fat with soybean oil lowered serum cholesterol by 16%. The primary outcome was first relapse (myocardial infarction, angina, sudden death). After 4 years, 62 of 199 in the soybean oil group had had a recurrent coronary event compared with 74 of 194 in the high saturated fat group; the difference, −18% (95% CI, −38 to 7), was not statistically significant.

The Finnish Mental Hospital Study compared a diet high in polyunsaturated fat, mainly from soybean oil, with a diet high in saturated fat in 1222 patients at 2 psychiatric hospitals.[30,36,37] In 1 hospital, the high polyunsaturated fat diet was given first, followed by the saturated fat diet; in the other hospital, the diets were given in the reverse order. Each diet period lasted 6 years. There were 2 cohorts. One comprised the entire patient populations of the 2 hospitals; 25% had evidence of CHD on an ECG, and 57% were women. The other cohort included only patients who had no evidence of CHD, that is, a primary prevention cohort. Women made up 44%. Serum cholesterol was 38 mg/dL (14%) lower on the high polyunsaturated fat diet than on the high saturated fat diet. Adherence was also demonstrated by 3-fold enrichment of linoleic acid in adipose tissue. In the mixed primary and secondary prevention cohort, CHD death, the primary outcome, was significantly lower by 41% (95% CI, −26 to −53) during the polyunsaturated than the saturated fat diet (Figure 1). In the primary prevention cohort, CHD death or myocardial infarction was the primary outcome, and the incidence was significantly lower also by 41% (95% CI, −17 to −58) during the polyunsaturated than during the saturated fat periods. In each hospital, CHD events were lower during the times when the polyunsaturated fat diet was given. Results were similar in men and women.

We performed a fixed-effects meta-analysis of these 4 core trials using the primary outcome chosen by each trial (Figure 2). This approach ensures that the results of the meta-analysis are based on prospectively defined primary outcomes, thereby having more validity than an alternative approach that redefines a new common outcome for all the component trials. This alternative approach would have a serious weakness, the selection of a new outcome that is post hoc and potentially influenced by researchers' bias. We included the entire Finnish trial population, primary and secondary prevention, women and men combined.

The results showed that lowering saturated fat and replacing it with vegetable oil rich in polyunsaturated fat, primarily soybean oil, lowered CHD by 29%. This effect on CHD is consistent with the effect of the experimental diet on serum cholesterol.[31] Each trial achieved a crucial element in clinical trial execution, producing and maintaining the required difference in diets as objectively documented by blood and tissue fatty acid biomarkers and serum cholesterol, that was needed to test the study aim. However, these trials were conducted in the 1960s, before widespread use of statins, when serum cholesterol levels were higher than now, as was the saturated fat content of the diet.

In addition to replacing dietary saturated fat with polyunsaturated fat, these clinical trials lowered dietary cholesterol. The cholesterol content of the diets was listed in 3 of the core trials.[20,22,30] We used the Keys[38] equation to estimate that the decreased cholesterol intake lowered serum cholesterol by ≈5 to 8 mg/dL, making up 15% to 20% of the total reduction in serum cholesterol. For example, in the Dayton et al[20] study, cholesterol intake decreased from 653 to 365 mg/d, and serum cholesterol decreased from 233 to 203 mg/dL, a 30-mg/dL difference, of which 6 mg/dL was accounted for by reduced dietary cholesterol. Because many foods that are high in saturated fat also contain cholesterol, the benefits to serum cholesterol lowering by reducing saturated fat will be augmented by consequent reduction in dietary cholesterol.

Noncore Trials on Replacing Saturated With Polyunsaturated Fat

In addition to the 4 core trials, several other trials aimed to test the hypothesis that replacing saturated with polyunsaturated fats reduces CHD.[7,23,32–35] We did not include these trials in our core group because they had a mixed dietary intervention in which polyunsaturated and carbohydrate replaced saturated fat and had insufficient duration, low adherence, few events, and/or serious flaws in study design. STARS (Saint Thomas Atherosclerosis Regression Study) was a 3.3-year trial that achieved its primary aim of reducing the severity of stenoses (blockages) in the coronary arteries.[7] The dietary treatment lowered saturated fat intake and replaced it with carbohydrates and polyunsaturated fat. The diet lowered serum cholesterol by 11%. CVD events (fatal CHD or nonfatal myocardial infarction) occurred in 2 of 27 participants in the diet group versus 5 of 28 in the control group.

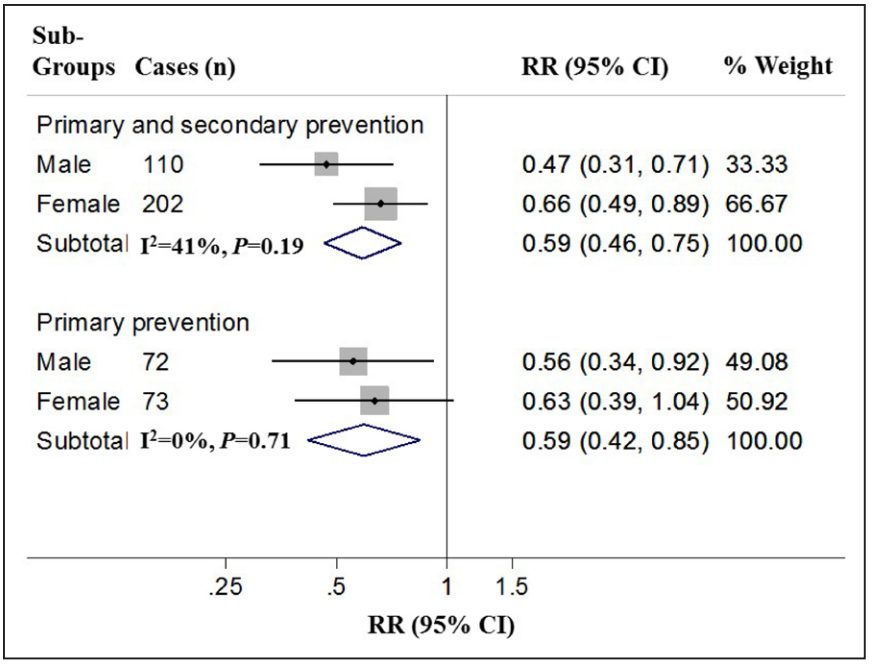

Figure 1. Finnish Mental Hospital Study.[30,36,37] Significant reduction in coronary heart disease (CHD) by replacing saturated with polyunsaturated fat. Results are shown separately for participants in the primary and secondary CHD prevention cohorts of the trial and participants who were in the primary CHD prevention cohort. Relative risks (RRs) of the primary CHD outcome, CHD death, were calculated on the basis of age-adjusted deaths rates, and results were pooled across sex by inverse-variance fixed-effects meta-analyses. CI indicates confidence interval. Meta-analysis by Drs Yanping Li and Jason H.Y. Wu.

The Welsh DART study (Diet and Reinfarction Trial)[23] compared the effect of fat advice with no fat advice on CVD during 2 years. The fat advice group reduced saturated fat from 15% to 11% of total calories, increased polyunsaturated fat from 7% to 9%, and increased carbohydrate intake from 44% to 46%. These changes fell well short of intended and produced only a 3.5% reduction in serum cholesterol. There were 8% fewer men with CHD death or nonfatal myocardial infarction in the fat advice group compared with the no-fat advice group, not statistically significant but similar to what is predicted from the small decrease in serum cholesterol.[31]

Houtsmuller et al[32] conducted a 6-year trial in patients with newly diagnosed diabetes mellitus that reduced saturated fat and replaced it with mainly polyunsaturated fat. Serum cholesterol decreased significantly by 7%. The high polyunsaturated, low saturated fat diet reduced the progression of diabetic microvascular disease, which was the primary outcome. CVD events were determined by electrocardiography; however, those reading the ECGs were not blinded to treatment assignment. For this reason, this trial was not included in the core group. The high polyunsaturated, low saturated fat group experienced significantly fewer CVD events, 8 of 51 versus 24 of 51, a 67% reduction, much greater than the 12% predicted by the modest lowering of serum cholesterol.

Rose et al[33] conducted a trial in male patients with CVD that replaced saturated fat with polyunsaturated

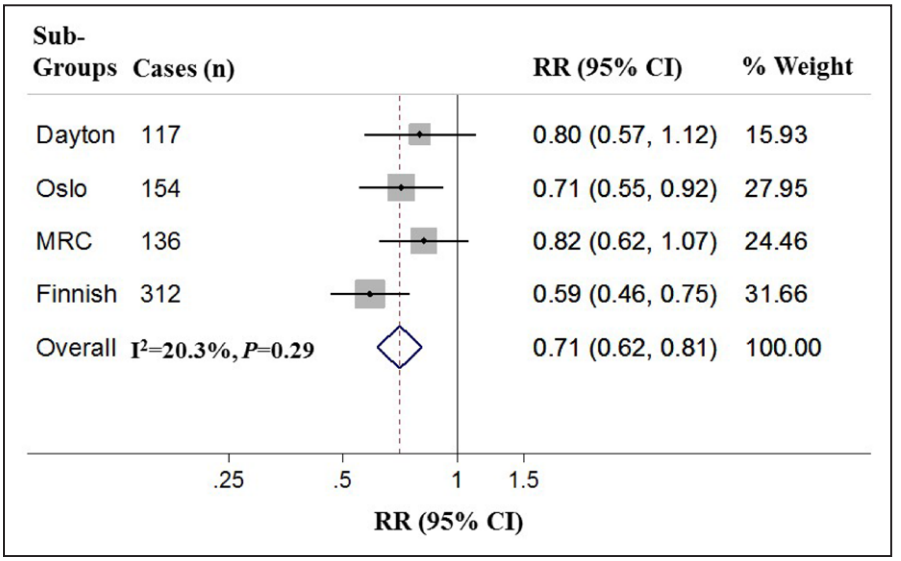

Figure 2. Meta-analysis of core trials on replacing saturated with polyunsaturated fat. Significant reduction in coronary heart disease (CHD). Relative risk (RR) of the primary CHD outcome of each trial. Findings across studies were pooled by inverse-variance fixed-effects meta-analyses. Risk reduction is the same with random-effects meta-analysis: RR, 0.71 (95% confidence interval [CI], 0.61–0.83). Data are from Dayton et al,[20] Oslo Diet-Heart Study,[21] Medical Research Council,[22] and Finnish studies.[30,36,37] Result from the Finnish trial used the total cohort (ie, patients who participated in the primary or secondary prevention cohorts). Meta-analysis by Drs Yanping Li and Jason H.Y. Wu.

corn oil. There were 26 patients in the control group and 28 in the corn oil group. The mean duration for receiving corn oil was 1.5 years. There were 12 cardiovascular events in the corn oil group versus 6 in the control group, not a statistically significant difference. The small number of participants and short duration of the trial excluded it from the core group.

The Minnesota Coronary Survey[34] compared high polyunsaturated with high saturated fat diets in patients hospitalized for mental illness. The participants were given the assigned diets only when they were patients in the hospital. Because hospitalization for mental illness became less common and less prolonged after the study started, as a national trend, the patients received the assigned diets intermittently, contrary to the intent of the researchers, and for a much shorter time than planned. The researchers originally enrolled 9570 participants in the trial and intended to study them for at least 3.6 years to be able to adequately test the effect of the diets. However, the trend toward outpatient treatment of mental illness resulted in ≈75% of the participants being discharged from inpatient care during the first year of the study. Only about half the remaining patients stayed in the study for at least 3 years. The average duration was only 384 days. The incidence of CHD events was similar in the 2 groups, 25.7 and 27.2 per 1000 person-years in the control and polyunsaturated fat groups, respectively. A recent reanalysis of this trial restricted to the participants who remained in the trial for at least 1 year also found no significant differences in CHD events or CHD deaths.[39] We excluded this trial from the core group because of the short duration, large percentage of withdrawals from the study, and intermittent treatment, which is not relevant to clinical practice. Another concern is the use of lightly hydrogenated corn oil margarine in the polyunsaturated fat diet. This type of margarine contains *trans* linoleic acid, the type of *trans* fatty acid most strongly associated with CHD.[40]

The Sydney Heart Study[35] was unique among the diet trials on CVD because a margarine high in *trans* unsaturated fat was a major component of the diet for participants assigned to the high polyunsaturated diet. When this trial was conducted, there was little recognition of the harms of *trans* unsaturated fat in partially hydrogenated vegetable oils, so the researchers inadvertently tested substitution of saturated with an even more atherogenic *trans* fat. As predicted from current knowledge about *trans* unsaturated fat, CVD events were higher in the experimental group. If anything, this trial confirmed the results of observational studies that also report higher CVD risk from results from regression models in which *trans* unsaturated fat replaced saturated fat.[41,42] We did not include this trial in our evaluation of the effects of lowering dietary saturated fat because *trans* fats are not recommended[3,13] and are being eliminated from the food supply.[43]

Two meta-analyses[9,16] analyzed the 4 core trials plus Minnesota,[34] STARS,[7] and DART.[23] Both meta-analyses showed a significant reduction in CVD of 19% by replacing saturated with polyunsaturated fat. Another systematic review and meta-analysis[10] included the Dayton et al study,[20] the Oslo Diet-Heart Study,[21] the Medical Research Council study,[22] the study by Houtsmuller et al,[32] the Rose et al study,[33] and the Sydney Diet Heart Study[35] and excluded the Finnish trial.[30,36,37] The Finnish trial was not included because it had 2 hospitals rather than at least 6 in the cluster randomization scheme, as required by the researchers conducting this meta-analysis.[10] In this group of trials, reduced saturated fat and increased polyunsaturated fat significantly lowered CVD events by 27% (see Table 9 in Reference 10). The extent of reduction in dietary saturated fat was significantly associated with the extent of decrease in CVD events among the trials. Reduction in serum cholesterol, as a consequence of reduced saturated fat and increased polyunsaturated fat, explained virtually all the variation among the trials in CVD event reduction.

The core trials reviewed in this section were started in the late 1950s and early 1960s. Readers may wonder why at least 1 definitive clinical trial has not been completed since then. Reasons include the high cost of a trial having upward of 20 000 to 30 000 participants needed to achieve satisfactory statistical power, the feasibility of delivering the dietary intervention to such a large study population, technical difficulties in establishing food distribution centers necessary to maintain high adherence for at least 5 years, and declining CVD incidence rates caused by improved lifestyle and better medical treatment. These linked issues, which must be managed to obtain a definitive result, remain the central considerations for dietary trials on CVD and indeed are the overarching reason why few of these trials have ever been done. Finally, by the 1980s, with rising rates of breast and colon cancer, the US government committed to conducting the WHI (Women's Health Initiative),[24] a trial that studied a diet aimed at decreasing total fat in the diet to 20% with the expectation that saturated fat would likewise be substantially decreased. Consequently, carbohydrates were increased in the diet. Details are discussed subsequently.

In summary, randomized controlled trials that lowered intake of saturated fat and replaced it with polyunsaturated vegetable oil reduced CVD events by ≈30%, similar to the reduction achieved by statin treatment.[31] Adding trials weakened by a short duration, low adherence, or use of *trans* unsaturated fat partially diluted the effect of the higher-quality core trials, but the results of meta-analyses that included both core and noncore trials still showed significant and substantial reduction in CVD when saturated fat is replaced with polyunsaturated fat.[9,10,16]

Low-Fat, High-Carbohydrate Diets

Few trials have studied the effect of reducing saturated fat and replacing it mainly with carbohydrates, without including with the diet other treatments such as antihypertensive or lipid-lowering medication. The British Medical Research Council[25] studied 252 men after myocardial infarction aiming to reduce total fat from 41% to 22% and maintaining it at 41% in the control group. The type of fat was similar in the high- and low-fat groups, mainly saturated fat from dairy products and meat. The low-fat, high-carbohydrate diet lowered serum cholesterol by 5%, less than expected from the planned reduction in saturated fat. The researchers remarked that the low-fat diet was unpleasant and difficult to tolerate. There were 48 CHD events in the control group compared with 46 in the low-fat group. The specific carbohydrate-containing foods in the low-fat diet group were not described except that sugar intake and skim milk were increased and biscuits and cakes were decreased.

DART, described previously,[23] lowered total fat from 35% to 31% by reducing saturated fat, replacing it partly with carbohydrates and partly with polyunsaturated fat. The reduction in CHD events, 8%, was not significant.

Originally designed as a diet study to prevent breast and colon cancer, the WHI tested the hypothesis that reducing all types of fats and replacing them with high-carbohydrate foods, particularly fruits and vegetables, decreases CVD.[24] Enrolled between 1993 and 1998 and conducted in postmenopausal women 50 to 79 years of age, this trial assigned 30 000 women at random to maintain their usual high-fat diet (37% of total energy intake) and 20 000 to a low-fat diet (20% of energy intake). They were followed up for 8 years. This trial was not a test of reduction purely in saturated fat because monounsaturated and polyunsaturated fats were also reduced to meet the primary dietary objective of decreasing total fat. The emphasis on reduction of all types of fat came from its primary aim to test the hypothesis that decreasing dietary fat of any kind reduces breast and colon cancer. The effect of this type of diet on CVD was a secondary aim. After 5 years, the low-fat diet group lowered LDL cholesterol by 4 mg/dL, an ≈3% reduction, similar to the British Medical Research Council trial[25] and DART.[23] Similar to those studies, the participants in the low-fat group did not achieve the goal for reducing dietary fat (24% after the first year and 29% after the eighth year compared with 35% and 37%, respectively, in the control group). Also like the earlier studies, the low-fat diet in the WHI had no significant effect on coronary events or stroke.

A systematic review and meta-analysis[10] identified 6 trials that reduced saturated fat, replacing it mainly with carbohydrates. In contrast to the favorable results of trials using polyunsaturated fat as the replacement macronutrient reported in the same article,[10] the low-fat, high-carbohydrate approach did not significantly reduce CVD events (relative risk, –7%; 95% CI, –21 to 8).

In summary, a dietary strategy of reducing intake of total dietary fat, including saturated fat, and replacing the fats mainly with unspecified carbohydrates does not prevent CHD. In contrast to trials of polyunsaturated fat, adherence to the low-fat regimen fell short of the intention, impairing the ability of the trials to test a biologically based or efficacy hypothesis. The authors of these and other dietary trials[20,23,25] remarked on the difficulty experienced by participants in adhering to and maintaining goals to reduce dietary total fat. Finally, we note that a trial has never been conducted to test the effect on CHD outcomes of a low-fat diet that increases intake of healthful nutrient-dense carbohydrates and fiber-rich foods such as whole grains, vegetables, fruits, and legumes that are now recommended in dietary guidelines.

CVD OUTCOMES: PROSPECTIVE OBSERVATIONAL STUDIES

Prospective observational studies of diet and disease refer to research in which large populations provide information on their diet, lifestyle, health, and other characteristics and behaviors at the beginning and then are followed up for many years for the occurrence of disease.[44] This research technique has several key advantages over randomized controlled trials but also important weaknesses. Compared with clinical trials, prospective observational studies include larger and potentially more representative populations and have longer durations. Most important, participants choose their own intake of foods and beverages and do not have to adapt their diet to randomized diet assignment; therefore, the problem of sustaining adherence has no relevance. Furthermore, prospective observational studies can update dietary information periodically during the follow-up period. Observational studies are much less expensive than randomized controlled trials (expressed as cost per participant or cost per hypothesis tested). However, the observational approach likewise has weaknesses. Participants who have a high intake of saturated fat may have dietary and nondietary characteristics that differ from those with low intake of saturated fat, and these differences could affect CVD, creating a confounding situation. Incomplete or inaccurate ascertainment of dietary components can affect associations with disease. Meticulous collection of diet and health information and the statistical methods used can reduce or even eliminate the influence of confounding to isolate the effect of the nutrient itself. In summary, randomized controlled trials and prospective

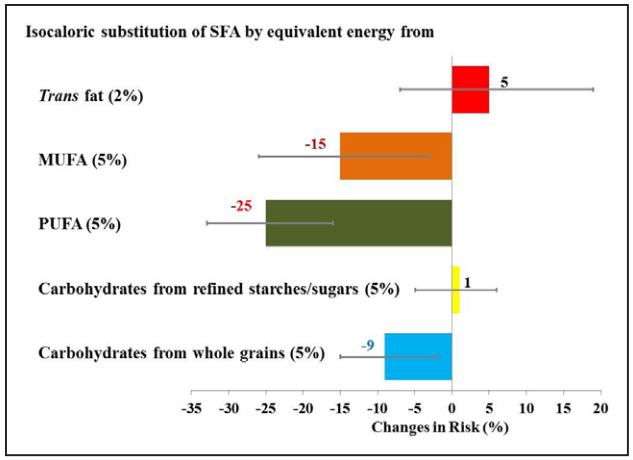

Figure 3. Replacement of saturated fat with other types of fat or carbohydrates.
Association with risk of cardiovascular disease in the Nurses' Health Study and Health Professionals Follow-Up Study. Multivariable adjustment. MUFA indicates monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; and SFA, saturated fatty acids. Modified from Li et al[18] with permission from The American College of Cardiology Foundation. Copyright © 2015, The American College of Cardiology Foundation.

observational studies are complementary research approaches. When the results are similar, the assumption of causality is strengthened between consumption of a dietary component and disease.

Fundamentally, methodology in observational analyses of diet and disease differs from that in analyses of biomarkers and genetic markers in relation to disease risk and is less familiar to scientists who work in other fields of epidemiology or in clinical trials. Observational studies in nutrition have special complexities, especially when studying foods or macronutrients such as fat and carbohydrates that make up a substantial portion of daily energy intake. For example, a low saturated fat intake occurs in the context of different dietary patterns, including low-fat, high-carbohydrate diets or Mediterranean diets high in unsaturated fat.

In North America and many European countries, the diets of people who eat a low-saturated fat diet typically are high in refined carbohydrates and low in unsaturated fats. For this reason, comparing CVD incidence in those with high and those with low saturated fat intakes primarily compares saturated fat with carbohydrates, most coming from refined grains, fruit juice, sweet desserts and snacks, sugar-sweetened beverages, and other foods. Well-publicized results of a meta-analysis reporting that saturated fat is not associated with CVD implicitly compare a high saturated fat diet with commonly eaten diets low in saturated fat and high in carbohydrate-containing foods made with refined carbohydrates and added sugars that themselves are associated with CVD.[15,16]

Further adding complexity, high-carbohydrate foods are very heterogeneous and may have beneficial or harmful associations with disease. For example, high-carbohydrate diets that include whole grains and cereal fiber are associated with lower rates of CVD, whereas refined grains and added sugars are associated with higher rates (Figure 3).[18]

Therefore, it is critical to the interpretation of findings in nutritional epidemiological studies that the contrast in dietary patterns between high and low saturated fat intake be well characterized. Simply comparing disease rates between people in a population who have low compared with high intake of saturated fat is fraught with potential for misinterpretation and misunderstanding.

Willett[44] developed a statistical framework for multivariable regression analysis that isolates effects of specific macronutrient exchanges. The method compares high saturated fat intake separately with high polyunsaturated fat, monounsaturated fat, *trans* unsaturated fat, and carbohydrates. The multivariable analysis equalizes other prognostic factors. In this way, the method simulates a randomized trial that compares 5 diets differing in type and amount of fat and carbohydrates. To determine the relationship of saturated fat with CVD outcomes in prospective observational studies, we used systematic reviews and meta-analyses published from 2009 to 2015,[12,17] the 2015 US Dietary Guidelines Advisory Committee Report,[13] and studies published after the report was released.[18,45] We considered studies that used multivariable regression analysis that isolates effects of specific nutrient exchanges.

The results showed that replacing 5% of energy intake from saturated fats with equivalent energy intake from polyunsaturated fats, monounsaturated fats, or carbohydrates from whole grains was associated highly significantly with a 25%, 15%, and 9% lower risk of CHD, respectively (Figure 3).[18] Replacing saturated fats with carbohydrates from refined starches/added sugars was not significantly associated with CHD risk (1% higher incidence). This pattern of results on dietary fats and CHD continued in analyses of total and cause-specific deaths; replacement of saturated fat by polyunsaturated fat (mainly linoleic acid) or monounsaturated fat was associated with lower rates of not only CVD death but also all deaths, deaths resulting from CVD, cancer, neurodegenerative disease, and lung disease (Figure 4).[45]

Key Points: Randomized Clinical Trials and Prospective Observational Studies on Replacement of Dietary Saturated Fat With Polyunsaturated or Monounsaturated Fat or Carbohydrates

- Four core randomized trials replacing saturated fat with polyunsaturated fat had at least 2 years'

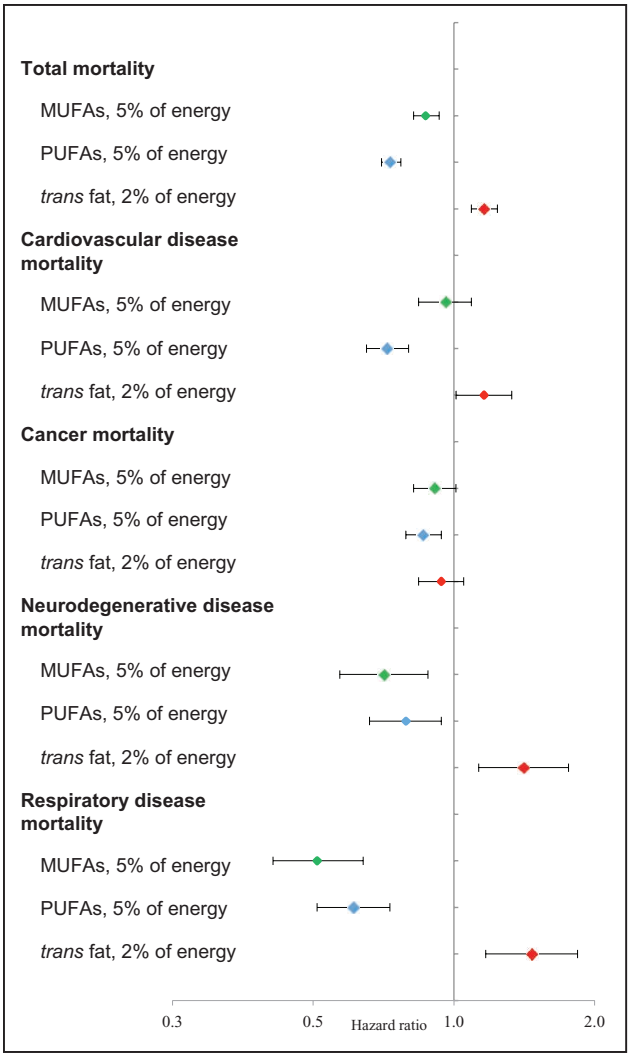

Figure 4. Replacement of saturated fat with other dietary fats.
Total and cause-specific mortality. Hazard ratio (95% confidence interval) for substituting energy from saturated fat by the same energy from specific types of fat. Nurses' Health Study and Health Professionals Follow-up Study. MUFA indicates monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids. Modified with permission from Wang et al.[45] Copyright © 2016, American Medical Association. All rights reserved.

duration, good adherence proven by blood or tissue levels of cholesterol and/or polyunsaturated fat, and standard outcome ascertainment. Meta-analysis showed a 29% reduction in CHD events.

- Six additional trials were not considered core trials because of short duration, low adherence, or nonstandard outcome ascertainment. However, meta-analyses that included several of these trials along with some or all of the core trials also found a significant reduction in CHD events on the polyunsaturated fat diet.
- The Sydney Diet Heart Study showed that using a margarine rich in *trans* unsaturated fat to replace saturated fat increased CHD events, confirming similar adverse results in epidemiological studies.
- Several trials that replaced saturated fat with carbohydrates did not show reduced CHD. Adherence was much less than expected in these trials.
- Prospective observational studies consistently found the following:
  - Lower risk of CHD when saturated fat was replaced with polyunsaturated or monounsaturated fat, more so for polyunsaturated than monounsaturated.
  - No decrease in risk of CHD when saturated fat was replaced with carbohydrates, especially carbohydrates from refined grains and added sugars. However, replacement with whole grains was associated with reduced CHD.
  - Lower risk of death resulting from CVD and all causes with replacement of saturated with polyunsaturated or monounsaturated fat.

DIETARY PATHOGENESIS OF ATHEROSCLEROSIS IN NONHUMAN PRIMATES

Because of their evolutionary similarities to human beings, nonhuman primate species were studied to determine the effects of diet on atherosclerosis. In these experiments,[5,46] to induce hypercholesterolemia and atherosclerotic lesion formation, one group of monkeys typically was fed lard or palm oil at 35% of their daily energy intake and dietary cholesterol to raise serum cholesterol levels into the 300- to 400-mg/dL range to model hypercholesterolemia in human beings at high risk for CHD. A second group of monkeys was fed a monounsaturated fat, high-oleic safflower oil, and a third group was fed a polyunsaturated fat linoleic acid–rich diet using safflower oil. Saturated fatty acids promoted higher LDL cholesterol concentrations and more coronary artery atherosclerosis. Linoleic acid lowered LDL cholesterol concentrations and decreased the amount of coronary artery atherosclerosis. In the oleic acid group, LDL cholesterol concentrations were lowered to an extent similar to that in the linoleic acid group, but paradoxically, the amount of coronary artery atherosclerosis was more like that in the saturated fat group.[5,47] In the oleic acid–rich diet group, the LDL particles of the monkeys were enriched in cholesteryl oleate and bound to arterial proteoglycans more avidly compared with the polyunsaturated fat diet group, an action that may be viewed as promoting atherosclerosis.[47,48] In humans as well, intake of high-oleic canola oil enriches LDL with cholesteryl oleate, but opposite to the findings in monkeys, this LDL has reduced bind-

ing to vascular proteoglycan, a potentially beneficial mechanism.[49] Atherosclerosis extent has consistently been positively correlated with high LDL proteoglycan binding affinity.[48,50]

Finally, a diet typical of the 1980s in the United States, high in saturated fat, fed to rhesus monkeys for 2 years increased serum cholesterol to 383 mg/dL and caused atherosclerosis that had complex pathological features similar to atherosclerosis in young human adults who died of trauma.[51] In contrast, a "prudent" diet recommended by the AHA to prevent CHD, low in saturated and high in polyunsaturated fat, produced lower serum cholesterol levels, 199 mg/dL, and less atherosclerosis.

In summary, in rhesus monkeys, African green monkeys, and cynomolgus monkeys, dietary saturated fat promoted coronary atherosclerosis during 1 to 5 years, whereas polyunsaturated fat reduced LDL cholesterol and coronary atherosclerosis.[5,6,46–51] The results strongly support the strong atherogenicity of saturated fatty acids through effects to raise LDL cholesterol concentrations compared with the effects of n-6 polyunsaturated fatty acids. Although monounsaturated fatty acids promoted atherosclerosis despite lowering LDL cholesterol, mechanisms related to LDL binding to proteoglycan may differ in humans. Generalization from these studies is limited by the high serum cholesterol levels produced by the atherogenic diets. Clearly, in >50 years of studies in nonhuman primates, saturated fat has proven to be atherogenic compared with polyunsaturated fat.

LDL CHOLESTEROL–MEDIATING DIETARY EFFECTS ON CVD

Dietary saturated and polyunsaturated fats are notable for their established opposing connections to LDL cholesterol levels. Reducing LDL cholesterol is a primary focus for preventive therapy. Replacing dietary saturated fat with unsaturated fat decreases LDL cholesterol levels, n-6 polyunsaturated fat more than monounsaturated fat.[4]

The LDL theory of atherosclerosis and CVD has support from the widest range of research studies[52]: studies that compare populations that vary in LDL cholesterol[52]; studies in single populations[52]; genetic studies of high LDL cholesterol caused by mutations impairing the action of LDL receptors to remove LDL from the blood circulation and lower LDL cholesterol levels[52]; studies of mutations in numerous other genes that affect LDL cholesterol by other mechanisms[53,54]; pharmacological studies that lower LDL cholesterol by decreasing cholesterol synthesis and increasing synthesis of LDL receptors by statins,[31] decreasing cholesterol absorption,[55] or inhibiting proprotein convertase subtilisin/kexin type 9 to increase LDL receptors[56]; studies of mutations in genes that interfere with assembly of LDL and its precursor very-low-density lipoprotein (VLDL) in the liver that decrease the amounts that are secreted into the circulation; correlations between LDL cholesterol and CVD reduction in meta-analyses of randomized clinical trials of statin and other LDL cholesterol–lowering treatments[31,55]; animal models that increase LDL cholesterol by diet or by genetic manipulation[6,57]; and studies of the processes by which atherosclerosis starts, progresses, and regresses in arterial vessels and cells.[57–59] Taking into consideration the totality of evidence, LDL cholesterol links saturated fat and its replacement macronutrients to CVD by very strong scientific evidence that satisfies rigorous criteria for causality.[60] Three independent guidelines committees rated this evidence as Level A, Strong.[3,13,61]

QUANTITATIVE EFFECTS OF DIETARY FATS AND CARBOHYDRATES ON LDL CHOLESTEROL

A systematic review and meta-regression analysis published last year identified and evaluated 84 randomized controlled trials including 2353 participants that studied the effect of dietary fats on LDL cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol.[4] The results were expressed as the amount of change in these lipids caused by a decrease in saturated fats of 1% of total daily calories and a 1% increase in polyunsaturated fat, monounsaturated fat, or carbohydrates. Polyunsaturated fat lowered LDL cholesterol by 2.1 mg/dL, monounsaturated fat by 1.6 mg/dL, and carbohydrates by 1.3 mg/dL (Figure 5, left). Replacing saturated with polyunsaturated fat is the most effective of these exchanges because the change from saturated to polyunsaturated fat combines a reduction in a LDL cholesterol–raising fat, saturated fat, with an increase in a LDL cholesterol–lowering fat, polyunsaturated fat. The independent effect of polyunsaturated fat is demonstrated by comparing it with carbohydrates: Replacing carbohydrates with polyunsaturated fat, 1% of daily energy, lowers LDL cholesterol by 0.9 mg/dL. The reductions in total or LDL cholesterol after diet change correlate well with the extent of reductions in CVD.[10]

The lifestyle report of the AHA and American College of Cardiology summarized studies that assessed the effect of dietary patterns on LDL cholesterol.[3] The report, taking an efficacy-based biological approach, reviewed "feeding trials" that composed complete diets and gave them to the study participants. These trials were DASH (Dietary Approaches to Stop Hypertension),[62] DASH-Sodium,[63] and DELTA (Dietary Effects on Lipoproteins

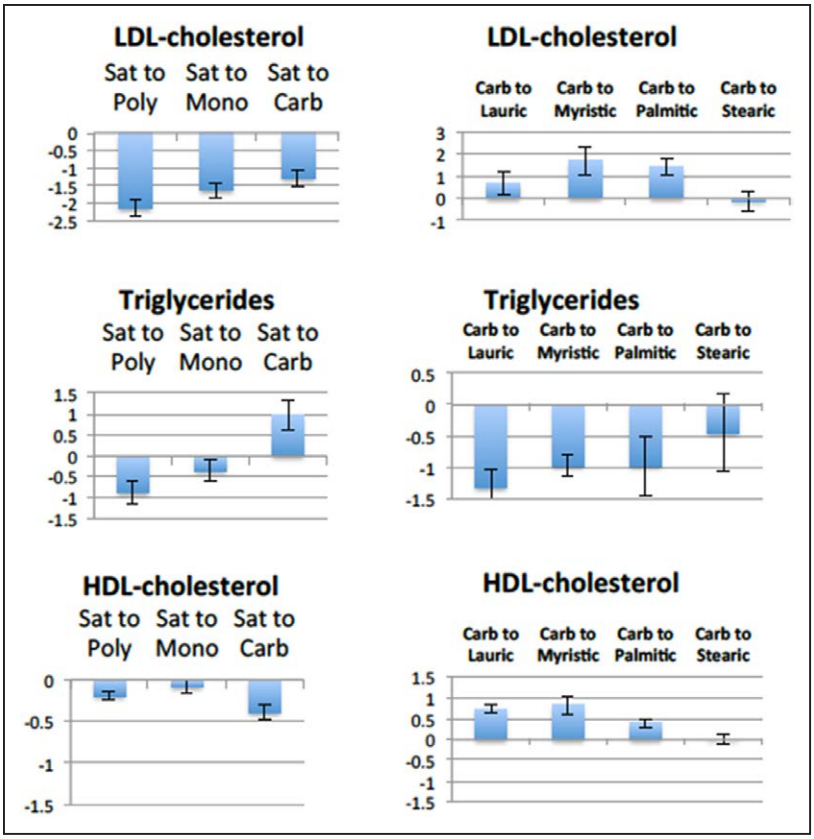

Figure 5. Effects of dietary fat and carbohydrates on blood low-density lipoprotein (LDL) cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol (mg/dL) in meta-regression analysis.
Left, Replacing saturated fat (Sat) with polyunsaturated fat (Poly) (n-6), monounsaturated fat (Mono), or carbohydrates (Carb). **Right**, Replacing carbohydrates with individual saturated fatty acids, lauric, myristic, palmitic, or stearic acid. Error bars show 95% confidence intervals. Data from Mensink.[4]

and Thrombogenic Activity).[64] Taken together, the trials found that a reduction in saturated fat in the context of dietary patterns intended to benefit lipid and other CVD risk factors lowered LDL cholesterol by amounts similar to those predicted by the meta-analysis.[4]

LDL Sizes

Some observational studies found that the concentration or proportion of large LDL predicts higher rates of CVD,[65–67] whereas other studies reported that small LDL predicts CVD[68,69] or both large and small LDLs predict CVD.[70,71] Still other studies found that LDL size, per se, does not predict CVD in a multivariable analysis that includes triglycerides or LDL concentration.[65,72–76] Dietary fat, in an equal combination of saturated and polyunsaturated replacing carbohydrates, increased the concentration of larger LDL and decreased smaller LDL sizes.[77] In another study, monounsaturated fat, replacing carbohydrates, reduced medium and small LDL, also shifting the distribution to the larger size.[78] Therefore, the effects of replacing carbohydrates with various kinds of fats qualitatively at least may be similar by increasing larger and decreasing smaller LDL sizes. Replacing saturated with monounsaturated fat lowered the concentrations of large, medium, and small LDL.[79] Replacing monounsaturated fat from olive oil with polyunsaturated fat from corn oil significantly lowered the concentrations of the total LDL cholesterol concentration, intermediate-density lipoprotein cholesterol, large LDL cholesterol, and nonsignificantly small LDL cholesterol.[80] Replacing *trans* unsaturated soybean oil with n-6 polyunsaturated corn oil lowered the concentration of small LDL.[81] In conclusion, this sparse set of findings suggests that replacement of saturated with monounsaturated or polyunsaturated fat reduces the concentration all sizes of LDL.

HDL CHOLESTEROL AND TRIGLYCERIDES: ADDITIONAL LIPID MEDIATORS

HDL Cholesterol

The aforementioned meta-analysis[4] also computed the effects of dietary fats and carbohydrates on 2 other blood lipid biomarkers of CVD risk, HDL cholesterol and triglycerides. A low HDL cholesterol level is associated with a high incidence of CVD in the context of a wide variety of concomitant conditions such as diabetes mellitus and obesity.[82] HDL can stimulate the removal of cholesterol from cells, including those involved in atherosclerosis, and can deliver the cholesterol to the liver where some of it may be secreted in bile and excreted, a process called reverse cholesterol transport.[83] However, unlike LDL cholesterol, genetic variation that affects HDL cholesterol is not associated with expected differences in

CVD unless LDL cholesterol or triglyceride is also affected by the genetic variants[84] or reverse cholesterol transport is impaired.[85] Still, these genetic studies, often called mendelian randomization, may not be capturing important loci for the protective effect of HDL that may be reflective in HDL cholesterol raising by dietary fats compared with carbohydrates. Although increases in HDL cholesterol by some pharmacological treatments have not decreased CVD,[86,87] this does not directly pertain to the effects of dietary fat because the underlying mechanisms of effects of drugs such as a cholesterylester transfer protein inhibitor and nicotinic acid are probably not the same as those affected by dietary fats and carbohydrates. The HDL field is working toward a functional approach to CVD risk prediction and treatment. For example, a small experimental study showed that consumption of saturated fat reduces the anti-inflammatory potential of HDL and impairs arterial endothelial function. In contrast, the anti-inflammatory activity of HDL improves after consumption of polyunsaturated fat.[88]

Replacing saturated fat with polyunsaturated or monounsaturated fat (1% daily energy exchanged) lowers HDL cholesterol slightly by 0.2 and 0.1 mg/dL (Figure 5, left).[4] Using carbohydrates as a replacement lowers HDL cholesterol more by 0.4 mg/dL. Carbohydrates also lower HDL cholesterol when replacing monounsaturated or polyunsaturated fats. Both low- and high-glycemic-index carbohydrates lower HDL cholesterol.[89,90]

Triglycerides

The plasma level of triglyceride is a well-established independent biomarker of CVD risk,[91] and triglyceride-rich lipoproteins have atherogenic properties. Triglyceride predicts CVD in a wide range of circumstances. Its association with CVD risk is partly attenuated by adjustment for HDL cholesterol, with which it is moderately correlated.[92] Genetic variation associated with lifelong low triglyceride levels is associated with a lower incidence of CVD.[90] Triglyceride is carried primarily within large lipoproteins, chylomicrons, and VLDL, which are also rich in cholesterol and like LDL can enter the arterial wall and stimulate atherosclerosis. These triglyceride-rich lipoproteins carry various atherogenic proteins such as apolipoprotein C-III, itself associated with atherosclerosis and CVD.[93]

Replacing 1% of daily energy intake from saturated fat with polyunsaturated or monounsaturated fat lowers triglyceride by 0.9 or 0.4 mg/dL, respectively (Figure 5, left),[4] perhaps more in those with hypertriglyceridemia.[91] Replacing the 1% saturated fat with 1% carbohydrates raises serum triglycerides by ≈1 mg/dL. Dietary carbohydrates raise plasma triglyceride levels by increasing the production by the liver of triglycerides and subsequent incorporation into VLDL.[91] The magnitude that dietary carbohydrates increase plasma triglyceride is similar whether the carbohydrate has a high or low glycemic index.[89]

INDIVIDUAL SATURATED FATTY ACIDS

The Mensink[4] meta-regression analysis determined the effects on blood lipids of replacing carbohydrates with the individual saturated fatty acids that are in common foods, including lauric, myristic, palmitic, and stearic acids. Lauric, myristic, and palmitic acids all had similar effects in increasing LDL cholesterol and HDL cholesterol and decreasing triglycerides when replacing carbohydrates (Figure 5, right).

Stearic acid makes up ≈20% of the fat in beef, 30% of the fat in pure cocoa (chocolate), and 10% to 15% in lard (pork fat) and lamb fat (Table). In contrast to the other saturated fatty acids, stearic acid does not increase LDL cholesterol or HDL cholesterol or decrease triglycerides when replacing carbohydrates (Figure 5, right). However, replacing stearic acid with unsaturated fat lowers LDL cholesterol.[4]

In summary, the common individual saturated fats raise LDL cholesterol. Their replacement with monounsaturated or polyunsaturated fats lowers LDL cholesterol. Differences in the effects of the individual fatty acids are small and should not affect dietary recommendations to lower saturated fat intake.

COCONUT OIL

A recent survey reported that 72% of the American public rated coconut oil as a "healthy food" compared with 37% of nutritionists.[94] This disconnect between lay and expert opinion can be attributed to the marketing of coconut oil in the popular press. The fatty acid profile of coconut oil is 82% saturated, about half lauric acid, and the rest myristic, palmitic, stearic, and short-chain fatty acids (Table). Lauric acid replacing carbohydrates increases LDL cholesterol but by about half as much as myristic and palmitic acids (Figure 5, right). Lauric acid increases HDL cholesterol about as much as myristic but more than palmitic acid. The net effect of increasing lauric acid and decreasing carbohydrates is a slight reduction in the ratio of LDL cholesterol to HDL cholesterol. However, as discussed earlier in this report, changes in HDL cholesterol caused by diet or drug treatments can no longer be directly linked to changes in CVD, and therefore, the LDL cholesterol–raising effect should be considered on its own. Furthermore, with respect to CVD, the informative comparison is between coconut oil and vegetable oils high in monounsaturated and polyunsaturated fats. A carefully controlled experiment compared the effects of coconut oil, butter, and safflower oil supplying polyunsaturated linoleic acid.[95]

Both butter and coconut oil raised LDL cholesterol compared with safflower oil, butter more than coconut oil, as predicted by the meta-regression analysis of individual dietary saturated fatty acids (Figure 5, right). Another carefully controlled experiment found that coconut oil significantly increased LDL cholesterol compared with olive oil.[96] A recent systematic review found 7 controlled trials, including the 2 just mentioned, that compared coconut oil with monounsaturated or polyunsaturated oils.[97] Coconut oil raised LDL cholesterol in all 7 of these trials, significantly in 6 of them. The authors also noted that the 7 trials did not find a difference in raising LDL cholesterol between coconut oil and other oils high in saturated fat such as butter, beef fat, or palm oil. Clinical trials that compared direct effects on CVD of coconut oil and other dietary oils have not been reported. However, because coconut oil increases LDL cholesterol, a cause of CVD, and has no known offsetting favorable effects, we advise against the use of coconut oil.

DAIRY PRODUCTS

Dairy fat is composed of 27% palmitic acid, 12% stearic acid, 9% myristic acid, and 3% lauric acid, for a total of 51% saturated fatty acids that raise LDL cholesterol compared with the unsaturated fatty acids (Table). Short-chain saturated fatty acids total 11%; monounsaturated, 26%; and polyunsaturated, 4%. Dairy fat also contains a very small amount of odd-chain fatty acids, 15:0 and 17:0, ≈0.5% to 1% of total fatty acids, and *trans* unsaturated fat, 4%, both made by bacteria in the ruminant gut. As we discuss subsequently, *trans* unsaturated fat made by ruminants has adverse effects on lipid risk factors similar to those of *trans* made industrially by partial hydrogenation.

Recent epidemiological studies measured blood levels of odd-chain fatty acids; one study found that they are associated with lower risk of CHD,[98] whereas another did not find such a relation.[99] It is not clear whether blood levels of odd-chain fatty acids represent intake of dairy fat or an effect of fat absorption and metabolism because the correlations between dairy fat intake and blood levels of odd-chain fatty acids (15:0, 17:0) are low (0.3).[99] Because of increasing consumption of low- and reduced-fat milk and other dairy products and decreasing consumption of full-fat dairy, especially whole milk, in the US population, the amount of dairy fat from low-fat compared with full-fat dairy is likely to have substantially increased. Therefore, dairy fat biomarkers may reflect both high- and low-fat dairy consumption patterns in the population. To the best of our knowledge, there are no biological mechanisms that link odd-chain fatty acids to protection against atherosclerosis and CVD.

For many years, there has been sporadic speculation that cheese is a unique food category, protective against CVD because it is manufactured by fermentation. To the best of our knowledge, no information from controlled studies supports the hypothesis that fermentation adds beneficial nutrients to cheese that counteract the harmful effects of its saturated fat. Recently, a clinical trial compared 3 diets, one with a high content of cheese, another with a high content of beef, and a third that was low in all types of fat, saturated, monounsaturated, and polyunsaturated.[100] The cheese and beef diets had higher amounts of saturated, monounsaturated, and polyunsaturated fatty acids. Neither the beef nor the cheese diet increased LDL cholesterol compared with the low-fat diet, as expected because of the counteracting effects of saturated and unsaturated fats on LDL cholesterol. Both the cheese and meat diets increased HDL cholesterol, consistent with the known effects of dietary fat to raise HDL cholesterol.[4] Therefore, the findings from this study do not support the hypothesis that cheese has special protective effects compared with beef on lipid risk factors for CVD.

Many controlled trials showed that dairy fat, often the major source of saturated fat in a study, increased LDL cholesterol compared with monounsaturated and polyunsaturated vegetable oils, reflecting the preponderance of saturated fatty acids, as reviewed previously in this report. Prospective observational studies found that the substitution of polyunsaturated fat for dairy fat, 5% of total daily calories, was associated with a 24% to 25% lower risk of CHD and stroke.[101] In contrast, substituting refined carbohydrates for dairy fat was not associated with reduced risk of CVD, whereas substituting carbohydrates from whole grains for dairy fat was associated with a 34% lower incidence of CHD and a 16% lower incidence of stroke. This analysis demonstrates again that it is essential to analyze the effects of unsaturated fats, refined carbohydrates, and whole grains separately to reach an informed and useful result for dietary advice.

In Finland, a successful nationwide health project to lower the very high rate of CHD mortality, started in 1972, had as a major goal the reduction in the high intake of saturated fat.[102] The project reduced intake of high-fat milk and butter, which lowered serum cholesterol by 13% in men and 18% in women. By 1992, CHD death rates decreased by 55% in men and 68% in women. Reduction in serum cholesterol accounted for ≈50% of the total reduction in CHD mortality.[103] Other dietary changes that may have contributed to the lower CHD mortality included increased fruits and vegetables, increased fish, decreased sugar, a shift from fatty to lean meats, and reduced sodium.

TRANS UNSATURATED FATS AND CVD

Trans unsaturated fatty acids are monounsaturated or polyunsaturated fatty acids containing at least 1 double bond in the *trans* configuration. There are 2 major types of *trans* fatty acids: naturally occurring found in meat and

milk of ruminant animals (eg, cattle and sheep), called ruminant *trans* fatty acids, and produced by chemical and enzymatic action for use in partially hydrogenated vegetable oils, called industrial *trans* fatty acids.[40,41] Both sources of *trans* fatty acids contain a range of fatty acid isomers, and there is considerable overlap. Food manufacturers have taken advantage of the low cost, long shelf-life, and the ability of *trans* fatty acids to withstand repeated heating and use partially hydrogenated vegetable oil in a variety of processed foods, including margarines, baked foods, and commercial deep-fried foods.

Clinical trials have consistently documented the adverse effects of *trans* fatty acids on the lipid risk factors for CVD. Replacement of calories from other types of fats with *trans* fatty acids raises LDL cholesterol, apolipoprotein B, triglycerides, and lipoprotein(a), as well as lowering HDL cholesterol and apolipoprotein A1.[104] Such effects are particularly large when *trans* fatty acids replace monounsaturated or polyunsaturated fatty acids but also occur when substituted for saturated fatty acids. The effects of *trans* fatty acids on blood lipids are potentially mediated through mechanisms including a reduction in the catabolism of LDL apolipoprotein B-100 and an increase in the catabolism of HDL apolipoprotein A-I, as well as enhancement of cholesteryl ester transfer protein activity.[105–107] Although most human trials were conducted with partially hydrogenated vegetable oil, emerging evidence suggest the ruminant *trans* fatty acids have similar adverse effects on blood lipids.[108–110]

Prospective observational studies have consistently concluded that higher total *trans* fatty acid intake is associated with elevated risk of CHD. A recent systematic review and meta-analysis of observational studies reported that higher intake of total *trans* fatty acid intake was associated with a 21% higher risk in total CHD (95% CI, 10–33; n=6 studies) and a 28% higher risk in CHD mortality (95% CI, 9–50; n=5 studies).[111] Although industrial *trans* fatty acids were consistently associated with total CHD and CHD death in observational studies, ruminant *trans* fatty acids were generally not.[112] The exact reason for these discrepant relationships remains unknown but may relate to the very low levels of ruminant trans fatty acids in these studied populations (mean intake, ≈0.7% of total energy),[112] differences in *trans* fatty acid isomers between ruminant and industrial *trans* fatty acids that have diverse biological effects, or confounding by the high amount of saturated fat in the major source of ruminant *trans* fatty acids.

In summary, the concordance between the adverse effects of *trans* fatty acids on lipid risk factors for CVD and the robust association of higher *trans* fatty acid intake with elevated CHD risk in observational studies provides the impetus for current policy actions of many local and national jurisdictions to reduce industrial *trans* fatty acids in the food supply.[113] Recognizing the need to act, in addition to requiring the *trans* fatty acid content of packaged foods to be listed on the Nutrition Facts label, the US Food and Drug Administration has recently revoked the generally recognized as safe status of partially hydrogenated vegetable oil, which should ensure further reductions in the population-level industrial *trans* fatty acid intake.[43]

N-3 (OMEGA-3) FATTY ACIDS

Polyunsaturated fatty acids exist in the n-3 or n-6 isomeric configuration. Both isomers are essential nutrients and have different biological effects. N-3 and n-6 fatty acids are not interconverted. Dietary n-6 polyunsaturated fatty acids, primarily linoleic acid, are much more prevalent than n-3 polyunsaturated fatty acids in vegetable oils and the total diet. α-Linolenic acid, a dietary n-3 polyunsaturated fatty acid, is present in soybean and rapeseed (canola) oil, walnuts, some green vegetables in very small amounts, chickens fed high–α-linolenic acid feed and their eggs, and grass-fed beef. Fish oil contains the very-long-chain n-3 polyunsaturated fatty acids, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid.

α-Linolenic Acid (Vegetable Omega-3)

A systematic review identified 4 randomized controlled trials that tested α-linolenic acid 2 to 6 g/d.[16] The Alpha-Omega trial tested the effect of α-linolenic acid 2 g/d compared with the same amount of oleic acid (both from 20 g margarine) in an older Dutch population with CHD.[114] There were ≈2400 participants in the α-linolenic acid and the oleic acid groups. After an average of 3.4 years of follow-up, the incidence of major cardiovascular events was 13.2% compared with 14.5% in the α-linolenic acid and control group, a 9% difference, not statistically significant. Two trials were conducted in Norway, one in 200 men who had CHD[115] and another in 13 400 men who were healthy and without CHD.[116] α-Linolenic acid 5 g/d supplied by flaxseed or linseed oil was tested and compared with sunflower oil, which has mainly n-6 linoleic acid. α-Linolenic acid did not significantly reduce CHD in either trial.

A meta-analysis of 7 prospective observational studies on dietary α-linolenic acid found an overall relative risk of 1.02 for CHD (nonsignificant).[16] However, there is consistent evidence that higher α-linolenic acid intake and higher blood levels of α-linolenic acid are associated with lower risk of fatal CHD.[117,118] α-Linolenic acid does not lower LDL cholesterol, but it has been shown to have antiarrhythmic properties in experimental studies.[119,120]

It has been proposed that α-linolenic acid affects CVD mainly in the low part of its range in the diet or when the background diet of the population under

study is almost completely devoid of eicosapentaenoic acid and docosahexaenoic acid.[121,122] This interesting hypothesis requires evidence from clinical trials.

In summary, randomized controlled trials and observational studies do not provide clear evidence that α-linolenic acid reduces the overall incidence of CVD, although higher intake of α-linolenic acid may reduce fatal CHD.

Eicosapentaenoic Acid, Docosapentaenoic Acid, and Docosahexaenoic Acid

The n-3 marine fatty acids eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid are present in fish and dietary supplements having a >10-fold range of n-3 fatty acid contents. High-dose prescription forms are also available to treat hypertriglyceridemia. The n-3 fatty acids contribute little energy to the daily diet and do not pertain closely to the topic covered by the present advisory. We refer readers who have interest in this complex topic to the AHA's library of guidelines, statements, and advisories.

MEDITERRANEAN DIETS, LYON HEART STUDY, AND PREDIMED

The Seven Countries Study kindled interest in Mediterranean diets.[11] Total fat intake was highest in Crete, Greece, at 43%, mainly from olive oil, where prevalence of CVD was lowest worldwide. Traditionally, Mediterranean diets had an abundance of plant foods, including vegetables, legumes, nuts, fruits, and grains, and fish.[123] As dietary patterns trended away from a traditional Mediterranean diet in Greece, individuals who maintained traditional diets experienced lower rates of death resulting from CVD, cancer, and all causes.[124]

The Lyon Heart Study[125] was a randomized controlled trial that provided α-linolenic acid 2 g/d as part of a Mediterranean diet intervention in 605 men with acute myocardial infarction. The Mediterranean diet replaced animal fat with polyunsaturated vegetable oil rich in α-linolenic acid; meat, butter, and cream were reduced, and fish, legumes, bread, fruits and vegetables were increased. A control group was assigned a low-fat diet. Mean follow-up was 27 months. Cardiovascular death or nonfatal myocardial infarction totaled 8 in the Mediterranean group and 33 in the control group, a significant difference. Although the researchers emphasized the α-linolenic acid component of the diet as contributing to the benefit, many other dietary changes occurred as part of the Mediterranean diet, making it impossible to determine to what extent α-linolenic acid contributed to the reduction in recurrent CHD.

The PREDIMED trial (Prevencion con Dieta Mediterranea) was a parallel-group, multicenter randomized trial in Spain conducted among 7447 men (age, 55–80 years) and women (age, 60–80 years) free of CVD at baseline and having either type 2 diabetes mellitus or 3 other risk factors for CVD.[126] They were assigned at random to a Mediterranean diet supplemented with 50 g extra virgin olive oil, a Mediterranean diet supplemented with 30 g nuts (half walnuts, one fourth almonds, and one fourth hazelnuts), or a reduced-fat control diet. Follow-up was 4 to 5 years. The primary end point, a composite of myocardial infarction, stroke, and death resulting from CVD, was lower significantly by 30% in the olive oil group and 29% in the nut group. The olive oil group increased intake of olive oil, partly replacing their usual kind of olive oil low in polyphenols with extravirgin olive oil high in polyphenols. The nuts group increased intake of α-linolenic acid and linoleic acid. Saturated fat intake was low, 9% of daily energy in all 3 groups during the trial. Monounsaturated fat intake was 21% to 22% in the Mediterranean groups compared with 19% in the reduced-fat group. Total fat was 41% in the Mediterranean and 37% in the reduced fat group. As intended by the researchers, the dietary changes reflected the aim to test a Mediterranean dietary pattern, not a specific alteration in dietary fat intake. Intake of fruits, vegetables, legumes, nuts, wine, and fish increased in the Mediterranean diet groups compared with the control diet group.

In summary, observational studies and 2 randomized clinical trials together suggest that a Mediterranean dietary pattern in which unsaturated fats predominate lowers the incidence of CVD.

CHILDREN

In 2012, the National Heart, Lung, and Blood Institute published the "Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents."[127] An expert panel reviewed the evidence building from the 2010 US Dietary Guidelines Advisory Committee Report.[128] Although evidence for dietary fat and cardiovascular risk in children was limited, key epidemiological studies provided the strongest data available. The Bogalusa Heart Study found that in children intake of animal fat, the major source of dietary saturated fat, was associated with higher body weight.[129]

The Cardiovascular Risk in Young Finns study (Young Finns) was a multicenter longitudinal cohort study of 3956 individuals 3 to 18 years of age in 1980 who had ongoing follow-up assessment of diet and blood lipids over 21 years.[130] Two major dietary patterns emerged, a "traditional" pattern including rye, potatoes, butter, sausages, milk, and coffee and a "health-conscious" pattern including vegetables, legumes and nuts, rye, cheese and other dairy products, and alcoholic beverages. In both men and women, at the end of follow-up, those following the traditional diet had higher levels of

total serum cholesterol and LDL cholesterol. Higher levels of LDL cholesterol in childhood predicted increased common carotid artery intima-media thickness, an indicator of atherosclerosis.[131]

The National Heart, Lung, and Blood Institute National Growth and Health Study recruited 2379 black and white 9-year-old girls in 3 different US cities and followed up their diets, growth, and development for a decade.[132] Girls who consumed a dietary pattern higher in fruits and vegetables, dairy products, and fiber-rich grains and lower in sugar, fried foods, burgers, pizza, and total fat for >10 years had lower body mass index, percentage of body fat, and waist circumference; these differences were significant for white girls.[132] Body mass index and central adiposity were correlated with LDL cholesterol.[133]

The STRIP trial (Special Turku Coronary Risk Factor Intervention Project for Babies) with >20 years of follow-up is the only randomized study that examined and reported improved long-term health effects from a multifactorial program that included a reduction in saturated fat starting in infancy compared with usual dietary intake and lifestyle among normal children from infancy through adolescence.[134,135] LDL cholesterol levels were lower in the intervention compared with the control group through 14 years of age, significant in boys but not girls.[135]

Likewise, the Diet Intervention Study in Children[136,137] was a randomized controlled trial designed to assess the safety and efficacy of a reduced-fat dietary intervention among prepubertal children with elevated LDL cholesterol levels (between the 80th and 98th percentiles) at baseline. A behavior-based, nutritionist-tailored intervention advocated adherence to a diet with 28% of energy from fat, <8% from saturated fat, and <9% from polyunsaturated fat. Saturated fat intake decreased in the intervention group compared with the control group throughout 7 years of follow-up.[136,137] LDL cholesterol was lower in the intervention compared with the control group, significant at 1 and 3 years but not at 5 and 7 years.

The PDAY study (Pathobiological Determinants of Atherosclerosis in Youth) provided crucial evidence that risk factors for developing CHD in adults were associated with atherosclerosis in men and women 15 to 34 years of age who died of accidents, homicide, or suicide.[138,139] Atherosclerosis was measured directly in the right coronary artery and abdominal aorta. The concentration of VLDL and LDL cholesterol was significantly directly associated and HDL cholesterol was inversely associated with early and intermediate lesions in both arteries. These results suggest that dietary factors that raise VLDL and LDL cholesterol produce atherosclerosis in teenagers and young adults.

Overall, these results suggest that reduced saturated fat intake within a healthful dietary pattern is feasible and effective for sustaining lower LDL cholesterol, a preventive effort against CVD in growing children.

CONCLUSIONS AND RECOMMENDATIONS

The key evidence to reduce saturated fat and replace it with polyunsaturated and monounsaturated fat is summarized below:

- Randomized clinical trials showed that polyunsaturated fat from vegetable oils replacing saturated fats from dairy and meat lowers CVD.
- A dietary strategy of reducing intake of total dietary fat, including saturated fat, and replacing the fats mainly with unspecified carbohydrates does not prevent CHD.
- Prospective observational studies in many populations showed that lower intake of saturated fat coupled with higher intake of polyunsaturated and monounsaturated fat is associated with lower rates of CVD and all-cause mortality.
- Saturated fat increases LDL cholesterol, a major cause of atherosclerosis and CVD, and replacing it with polyunsaturated or monounsaturated fat decreases LDL cholesterol
- Replacing saturated with polyunsaturated or monounsaturated fat lowers blood triglyceride levels, an independent biomarker of risk for CVD.
- Replacing saturated with polyunsaturated fat prevents and regresses atherosclerosis in nonhuman primates.
- Overall, evidence supports the conclusion that polyunsaturated fat from vegetable oils (mainly n-6, linoleic acid) reduces CVD somewhat more than monounsaturated fat (mainly oleic acid) when replacing saturated fat.

Evidence has accumulated during the past several years that strengthens long-standing AHA recommendations to replace saturated fat with polyunsaturated and monounsaturated fat to lower the incidence of CVD. Reduction in total dietary fat or a goal for total fat intake is not recommended. This shift from saturated to unsaturated fats should occur simultaneously in an overall healthful dietary pattern such as the DASH or Mediterranean diet as emphasized by the 2013 AHA/American College of Cardiology lifestyle guidelines and the 2015 to 2020 Dietary Guidelines for Americans.

FOOTNOTES

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

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*Modest.

†Significant.

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EXHIBIT D

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February 12, 2020

Via United States Mail

Glanbia Performance Nutrition (Manufacturing), Inc.
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KSF Acquisition Corporation d/b/a SlimFast
11780 U.S. Highway One, Suite 400 N
Palm Beach Gardens, FL 33408

Re: Notice and Demand Letter Pursuant to U.C.C. § 2-607; N.Y. G.B.L. §§ 349, 350; and all other applicable laws

To Whom It May Concern,

This letter serves as a preliminary notice and demand for corrective action by KSF Acquisition Corporation d/b/a SlimFast ("Defendant," "SlimFast," or "You") arising from breaches of warranty on behalf of our client, Gregory Maroney, and a class of all similarly situated purchasers of SlimFast Keto MCT Oil dietary supplement. This letter serves as notice pursuant to U.C.C. § 2-607(3)(a) concerning the breaches of express and implied warranties described herein. This letter additionally serves as notice of violations of all applicable consumer protection laws, including, but not limited to, New York General Business Law §§ 349 & 350.

You have participated in the manufacture, marketing, and sale of SlimFast Keto MCT Oil (the "Product"). SlimFast Keto MCT Oil is marketed and sold as a dietary supplement containing "100% Pure Coconut Oil." The front of the Product's label represents "Clinically Proven Lose Weight & Keep It Off" (the "Misrepresentations"). But that representation is not true. A 2018 peer-reviewed study[1] found "no evidence of difference in…mean weight, BMI, [or] per cent body fat" associated with the use of coconut oil. And far from being "clinically proven" to cause weight loss, a 2015 Meta-Analysis[2] concluded that "further research is required…to confirm the efficacy of MCT" because "many trials lacked sufficient information" and "commercial bias was detected." Indeed, the American Heart Association[3] "advise[s]

[1] Khaw, Kay-Tee et al. Randomised trial of coconut oil, olive oil or butter on blood lipids and other cardiovascular risk factors in healthy men and women. BMJ Open. 2018; 8(3): e020167.

[2] Mumme, Karen et al. Effects of Medium-Chain Triglycerides on Weight Loss and Body Composition: A Meta-Analysis of Randomized Controlled Trials. Journal of the Academy of Nutrition and Dietetics, Volume 115, Issue 2, 249 – 263.

[3] Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association (2017).

against the use of coconut oil" because it is "high in saturated fat…and has no known offsetting benefits." Accordingly, the Misrepresentations made on the Product's labeling are false and misleading.

Mr. Maroney purchased and used SlimFast Keto MCT Oil in reliance on the Misrepresentations, which Defendant expressly warranted. And Defendant breached these express warranties for the reasons stated herein. *See* U.C.C. § 2-313.

Defendant's conduct also constitutes a deceptive business practice and false advertising under all applicable consumer protection laws, including, but not limited to, New York General Business Law §§ 349 & 350.

Mr. Maroney is acting on behalf of a class defined as all persons in the United States who purchased SlimFast Keto MCT Oil. Mr. Maroney also seeks to represent a subclass of all persons in the state of New York who purchased SlimFast Keto MCT Oil.

To cure these defects, we demand that you (1) cease and desist from further sales of mislabeled SlimFast Keto MCT Oil; (2) issue an immediate recall of mislabeled SlimFast Keto MCT Oil; and (3) make full restitution to all purchasers of SlimFast Keto MCT Oil.

We further demand that you preserve all documents and other evidence which refer or relate to any of the above-described practices including, but not limited to, the following:

1. All documents concerning the design, development, supply, production, extraction, and/or testing of SlimFast's Keto MCT Oil;

2. All documents concerning the advertisement, marketing, or sale of SlimFast Keto MCT Oil;

3. All documents concerning communications with any retailer involved in the marketing or sale of SlimFast Keto MCT Oil;

4. All documents concerning communications with purchasers of SlimFast Keto MCT Oil;

5. All documents concerning communications with federal or state regulators; and

6. All documents concerning the total revenue derived from sales of SlimFast Keto MCT Oil in the United States.

If you contend that any statement in this letter is inaccurate in any respect, please provide us with your contentions and supporting documents promptly.

We are willing to negotiate to attempt to resolve the demands asserted in this letter. If you wish to enter into such discussions, please contact me right away. If I do not hear from you

promptly, I will take that as an indication that you are not interested in doing so.

Very truly yours,

Joseph I. Marchese